

**NEUROENDOCRINE ALTERATIONS IN
CHRONIC FATIGUE SYNDROME.**

**BY
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**A thesis submitted for the degree of Doctor of Philosophy
in the Department of Neurology, Faculty of Medicine,
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DECLARATION

I hereby declare that the work presented in this thesis is original and was conducted solely by the author, except where collaboration with others is acknowledged.

DEDICATION

To my parents.

**Thank you for the love,
prayers, encouragement and
sacrifice that enabled me
to go through this study.**

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ABBREVIATIONS

ACh	Acetylcholine
AChE	Acetylcholinesterase
ACTH	Adrenocorticotropin hormone
ADX	Adrenalectomy
AMP	Adenosine monophosphate
ANOVA	Analysis of variance
ATP	Adenosine triphosphate
AVP	Arginine vasopressin
CAT	Choline acetyltransferase
CDC	Centers for Disease Control
CFS	Chronic fatigue syndrome
cGMP	Cyclic guanine monophosphate
CNS	Central nervous system
COMT	Catechol-O-methyltransferase
CPK	Creatine phosphokinase
CRF	Corticotropin releasing factor
CRH	Corticotropin releasing hormone
CRNA	Complementary RNA
Cu	Copper
DA	Dopamine

DAG	Diacylglycerol
D ₂ receptor	Dopamine receptor
DEX	Dexamethasone
DNA	Deoxyribonucleic acid
DOPA	Dihydroxyphenylalanine
EA	Early antigen
EBV	Epstein Barr virus
ER	Endoplasmic reticulum
ESR	Erythrocyte sedimentation rate
FSH	Follicle stimulating hormone
G	Nucleotide regulatory protein
GABA	Gamma aminobutyric acid
GABA-T	GABA α oxoglutarate transaminase
GAD	Glutamic acid decarboxylase
GH	Growth hormone
GHRH	Growth hormone releasing hormone
G _i	Inhibitory nucleotide regulatory protein
GnRH	Gonadotropin releasing hormone
GR	Glucocorticoid receptor
G _s Protein	Stimulatory nucleotide regulatory protein
HHV	Human herpes virus

HIAA	Hydroxyindoleacetic acid
HIV	Human immune deficiency virus
HLA	Human leucocytic antigen
HPA	Hypothalamic-pituitary-adrenal axis
HTL-V	Human T cell lymphotropic virus
5-HT	5-hydroxytryptamine
IgA	Immunoglobulin A
IgD	Immunoglobulin D
IgG	Immunoglobulin G
IGF	Insulin-like growth factor
IgM	Immunoglobulin M
IP ₃	Inositol triphosphate
IPSP	Inhibitory postsynaptic potential
LCMV	Lymphocytic choriomeningitis virus
LH	Luteinizing hormone
LHRH	Luteinizing hormone releasing hormone
MHC	Major histocompatibility complex
MHPG	Methoxy hydroxyphenethyleneglycol
MR	Mineralocorticoid receptor
mRNA	Messenger RNA
NA	Noradrenaline
NE	Norepinephrine

NK	Natural killer cell
NMR	Nuclear magnetic resonance
NREM	Non-rapid eye movement
PCR	Polymerase chain reaction
PIP ₂	Phosphatidylinositol diphosphate
PLC	Phospholipase C
PKC	Protein kinase C
PRF	Prolactin releasing factor
PRL	Prolactin
REM	Rapid eye movement
Ri	Inhibitory receptor
RNA	Ribonucleic acid
Rs	Stimulatory receptor
SEM	Standard error of mean
SRIF	Somatotropin release-inhibiting factor
SS	Somatostatin
SSADH	Succinic semi-aldehyde dehydrogenase
TRH	Thyrotropin releasing hormone
TSH	Thyroid stimulating hormone
VIP	Vasoactive -intestinal peptide
VMA	Vanillylmandelic acid

α	Alpha
β	Beta
Δ	Delta

PUBLICATIONS

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SUMMARY

Chronic fatigue syndrome (CFS) is an illness that occurs worldwide. Over the years the syndrome has had various labels, ranging from the Victorian one of neurasthenia to the putative neurological diagnosis of benign myalgic encephalomyelitis (Acheson 1959). A working case definition was published by the Centers for Disease Control in 1988 (Holmes et al) and revised in 1994 (Fukuda et al).

In the initial studies of patients with CFS, impaired activation of hypothalamic-pituitary-adrenal (HPA) axis (Demitrack et al 1991) and monoaminergic dysfunction (Demitrack et al 1992; Bakheit et al 1992) was reported. Dynamic neuroendocrine challenge tests provide one of the only methods for examining neurotransmitter function *in vivo* in humans (Checkley 1980). In the following studies these tests were used to examine different neurotransmitter systems and their influence on the functional activity of the HPA, hypothalamic-somatotroph and hypothalamic-prolactin axes.

In the first study, serotonin (5-HT) function in CFS was evaluated using the specific 5-HT_{1A} receptor agonist, buspirone. It was found that prolactin responses to this agent were augmented in patients with chronic fatigue syndrome compared to healthy controls. This finding does not support the view that CFS is a form of depressive disorder because in depression, prolactin responses to this 5-HT_{1A} agonist (Bakheit et al 1992) and to d-fenfluramine (Cleare et al 1995) are blunted reflecting decreased

5-HT neurotransmission. The study is therefore of importance in suggesting that CFS unlike depression, may be associated with increased 5-HT function.

The second study is based on the hypothesis that abnormalities in the HPA-axis (Demitrack et al 1991) arise from a disturbance in serotonergic neurotransmission. ACTH and cortisol responses to the selective 5-HT_{1A} receptor agonist ipsapirone were examined: no differences in baseline ACTH and cortisol levels were found but in CFS, there was significant attenuation of ACTH release.

Study 3 was designed to demonstrate the functional integrity of the hypothalamic-somatotroph axis and its interaction with the HPA-axis. Growth hormone (GH) responses to dexamethasone were examined in two phases, before and after the administration of metyrapone, an inhibitor of 11- β hydroxylation, given to block steroid synthesis and upregulate brain steroid receptors. Blunted responses were recorded in each phase in patients with CFS and depression compared to healthy controls. It is concluded that the abnormality found is compatible with a decrease response and/or a lack of plasticity in cerebral glucocorticoid receptors.

In study 4, noradrenergic function was examined by measuring GH responses to the monoamine reuptake inhibitor, desipramine; blunted responses were found in patients with CFS, compared to healthy individuals. It is concluded that the attenuated response is due to decreased sensitivity of the α_2 - postsynaptic adrenoceptor, secondary to a hyperadrenergic state, indicating that central noradrenergic function is decreased in patients with CFS.

In study 5, growth hormone responses to anticholinesterase, pyridostigmine were examined: augmented responses were found in the CFS group, compared to the healthy individuals. This enhanced response reflects increased sensitivity of acetylcholine (ACh) neurotransmitter function in CFS.

In studies 6 & 7, growth hormone responses to the dopamine agonist, bromocriptine, and to the GABA (B) agonist, baclofen, were measured in order to assess dopamine (DA) and GABA neurotransmitter function. The responses in patients with CFS were the same as in the healthy controls. It is concluded that dopamine (DA) and GABA neurotransmitter systems are not involved in the pathophysiology of CFS.

In summary, the neuroendocrine investigations revealed positive findings of a) upregulation of 5-HT receptors with increased 5-HT function, b) impaired activation of the HPA-axis to serotonin input, c) cerebral steroid receptor resistance and d) reciprocal dysfunction in noradrenaline (NE) and acetylcholine (ACh)-mediated endocrine responses. These findings suggest that CFS is an organic illness with definite neuroendocrine abnormalities. Some of the neuroendocrine abnormalities are similar to those reported in depression, suggesting the reason why these two illnesses may share common symptoms.

GENERAL OVERVIEW OF CHRONIC FATIGUE SYNDROME

1.1 DEFINITION

Fukuda et al (1994) described a case definition of chronic fatigue syndrome (CFS) from the Centers for Disease Control (CDC) Atlanta, USA, revising the previous working model published in 1988 (Holmes et al 1988). According to the new definition, CFS is :-

- A. Clinically evaluated, unexplained, persistent or relapsing chronic fatigue that is of new or definite onset; is not the result of ongoing exertion; is not substantially alleviated by rest; and results in substantial reduction in previous levels of occupational, educational, social or personal activities.
- B. The concurrent occurrence of four or more of the following symptoms, all of which must have persisted or recurred during six or more consecutive months of illness and must not have predated the fatigue:
 - a. Self-reported impairment in short-term memory or concentration if severe enough to cause substantial reduction in previous levels of occupational, educational, social or personal activities.
 - b. Sore throat.
 - c. Tender cervical or axillary lymph nodes.
 - d. Muscle pain.
 - e. Multi-joint pain without joint swelling or redness.
 - f. Headaches of a new type, pattern, or severity.
 - g. Unrefreshing sleep.
 - h. Postexertional malaise lasting more than 24 hours.

This Working Case Definition, has to be used for research in clinical practice until more is known about the exact pathogenesis of CFS.

In the United Kingdom several different definitions were used, prior to the American CDC (1994) case description. United Kingdom researchers met in Oxford in 1990 for the purpose of proposing a working case definition for the United Kingdom (Sharpe et al 1991). A number of clinical syndromes had been described in the past which included chronic infectious mononucleosis (Isaacs, 1948), Icelandic disease (Sigurdsson 1950), Royal Free disease (Acheson 1955), benign myalgic encephalomyelitis (Galpine and Brady, 1957), epidemic neuromyasthenia (Henderson and Shelokov 1959), postviral fatigue syndrome (Behan et al 1985), fibrositis fibromyalgia (Pritchard 1988; Yunus 1989) and chronic fatigue syndrome (Holmes et al 1988).

The UK experts proposed division into two broad groups i.e. - Chronic Fatigue Syndrome, and Postinfectious Fatigue Syndrome.

CFS was characterized by the following features:

- a. Fatigue must be the principal symptom.
- b. The syndrome must be definite in onset.
- c. Be severe, disabling and have an effect on physical and mental (cognitive) functioning.
- d. Fatigue must have been present for more than 50% of the time for at least six months.
- e. Symptoms, including myalgia, mood and sleep disturbances could be present.
- f. Patients with medical conditions known to produce chronic fatigue and patients with a current diagnosis of schizophrenia, manic depressive illness, substance abuse, eating disorders or proven organic brain disease, were to be excluded. However, other psychiatric disorders such as depressive illness and anxiety disorders were not grounds for exclusion.

Postinfectious syndrome must:

Fulfil criteria for CFS and have in addition

- a Definite evidence of infection at onset or presentation.
- b The syndrome had to be present for a minimum of six months after the onset of infection.
- c The infection had to have been corroborated by laboratory evidence.

In 1988, in America Holmes and colleagues at the CDC in Atlanta attempted to define the syndrome. To meet their case definition for CFS, a case must fulfil both major and minor criteria (Holmes et al 1988).

Major Criteria:-

- a. The new onset of persistent or relapsing, debilitating fatigue in a person without a previous history of such symptoms that did not resolve with bed rest and that was severe enough to reduce or impair average daily activity to less than 50% of the patient's premorbid activity level for at least six months.
- b. Exclusion by a thorough evaluation based on history, physical examinations, and appropriate laboratory tests, of other clinical conditions that might produce similar symptoms.

Minor Criteria:-

Symptoms

a, Mild fever (oral temperature between 37.6°C and 38.6°C if measured by the patient, or chills). b, Sore throat. c, Painful lymphadenopathy (anterior or posterior cervical and axillary distribution). d, Unexplained generalised muscular weakness. e, Muscle discomfort or myalgia. f, Prolonged (more than 24 hours) generalised fatigue after previously tolerated levels of physical activity. g, Headaches. h, Migratory arthralgia without joint swelling or redness. i, Neuropsychiatric complaints (one or more of the following - photophobia, transient visual scotomata, forgetfulness, excessive irritability, confusion, difficulty thinking, depression and inability to concentrate). j, Sleep disturbance. k, Main symptom complex developing over a few hours to a few days.

Signs:-

a, Lymphadenopathy. b, Nonexudative pharyngitis. c, Low grade fever.

For a diagnosis of CFS to be made the patient must have fulfilled two major criteria and either eight of the eleven symptoms or have six symptoms and two signs.

Others in Australia (Lloyd et al 1990) designed yet another definition. According to them this syndrome requires the presence of the following features.

- a. Chronic persisting or relapsing fatigue causing significant disruption of usual daily activities that has been present for more than six months.
- b. Postexertional fatigue.
- c. Neuropsychiatric (cognitive) dysfunction including new onset of impairment of short term memory and concentration.

d. No alternative diagnosis was reached by history, physical examination or laboratory investigations over a six month period.

Behan and Bakheit (1991) have also given a clinical description of the illness with a differential diagnosis. The older clinical works on the syndrome did not set out a clear cut definition. For example, Acheson in his classical paper (Acheson 1959) while discussing the various outbreaks of this disorder, did not mention the fatigue but showed that all the patients shared the following characteristics: a) headaches; b) myalgia; c) paresis; d) symptoms or signs other than paresis suggestive of damage to the brain, spinal cord or peripheral nerves; e) mental symptoms; f) low or absent fever in most cases; h) no mortality. In addition, there was a) a higher frequency in women; b) a predominantly normal cerebrospinal fluid; and c) relapses were frequent in all of the outbreaks. Later, as knowledge of the illness grew, physicians concentrated on the fatigability and muscle pain (Shelokov 1972). It is now readily accepted that the term "chronic fatigue syndrome" will, until more is known of its exact pathogenesis, be the correct term, and the CDC 1994 Working Case Definition will be used as the operative definition.

1.2 A CRITIQUE OF PRESENT DEFINITIONS

The working case definition for CFS is based entirely on symptoms which cannot be measured objectively. Complaints can be exaggerated, coloured or magnified, particularly if the patient is psychoneurotic. The patients have to tell the truth. Physical signs are not very reliable since non-exudative pharyngitis is a common finding in smokers, singers and those exposed to dust. Lymphadenopathy, particularly in the cervical region, can merely represent recent or chronic infection in the head and neck. Lymph node enlargement in the posterior cervical area can persist for a long time after exposure to rubella. Fever is the only physical criteria which can

be given some consideration, provided it is noted by a reliable person on more than one occasion: one reading at the time of examination is not sufficient.

One of the clinical characteristics of the illness is that the majority of patients have an acute onset with a flu-like illness, accompanied by upper respiratory tract symptoms or gastroenteritis (Acheson 1959; Holt 1965). The illness has also been observed in farmers and agricultural workers exposed to sheep dips and insecticides containing organophosphates (Behan and Haniffah 1994). Cases have also been reported after gastroenteritis due to ciguatera poisoning (Gillespie et al 1986). Despite these observations there is no mention of precipitating factors in the present definition.

Since the CDC diagnosis is one of exclusion, one is at loss to know which investigations are necessary to rule out the many diseases causing fatigue, including malignancy, autoimmune diseases, chronic or subacute bacterial, fungal and parasitic disease, disease related to HIV infection, anemia, metabolic or neuromuscular disease, drug dependency or abuse, and chronic pulmonary, cardiac, gastrointestinal, hepatic, renal or hematologic disorders.

In the present definition, the cut-off time is six months which is not long enough to exclude carcinoma on the basis of history alone. A rating scale should be used with regard to symptoms since the more symptoms that are, the less likely is the condition to be organic, and less extensive investigations are required.

Another characteristic of the disease is that most of these patients have a tendency to maintain a meticulous chronologic symptom diary and are inclined to be hypergraphic. Shafran (1991) feels this fact should also be incorporated into the definition. They also stress that a history of the patient having consulted many

physicians previously (as such patients are often referred from physician to physician), should also be included.

Characteristics of exertion have not been dealt with in great detail. One should realise that exertion and exercise may precipitate other disorders presenting as fatigue, for example McArdle's disease and acid maltase deficiency. Measurement of exercise is of particular importance in these patients, particularly those who claim disability benefit and put claims to their insurance companies.

A lag period is not included in the definition. Fatigue may not appear immediately after exertion. Similarly where rest is referred to, its duration and quality are not defined (Armon and Kurland 1991).

The major criteria in the CDC 1988 definition exclude all those who cannot afford to reduce their average daily activity to less than 50% due to personal and professional demands. Quantification of a 50% reduction is very difficult, if not impossible.

The psychiatric evaluation used to exclude previous and present psychopathology is not clearly defined (Armon and Kurland 1991). There is no mention of exclusion of those who have a history of psychiatric illness but have not consulted a psychiatrist for precise diagnosis. Again there is no mention of personality disorders. Is it necessary for every patient to be seen by a psychiatrist in order to exclude psychiatric disorders, when 50% of the patients have no psychiatric illness whatsoever and standardisation of the diagnosis among psychiatrists is so controversial (Nathan et al 1969).

For epidemiologic purposes, where a questionnaire is used, the CDC definition does not work well since it refers to physical signs as well as symptoms. In order to

confirm such physical signs, each patient should be examined by a physician. The definition therefore cannot be relied on for epidemiological studies (Grufferman 1991).

A number of papers have drawn attention to the fact that the illness begins acutely, in as many as 85% of cases (Komaroff and Buchwald 1991). The working case definition of the CDC 1988 tends to ignore this important point but it is one which argues strongly for an organic aetiology. Since the illness may consist of heterogenous groups of patients (Wessely and Thomas 1989; Lane et al 1995) one of the purposes of the revised CDC 1994 case definition should be to define a patient group whose heterogeneity is reduced.

In all four definitions, as might be expected, there are similarities. All are agreed that the duration of fatigue should be six months; however, as seen from Table 1 (modified after Bates et al 1994 a) there is disagreement on the type or quality of fatigue severity. Fatigue, in both CDC definitions is required to be of new onset while in the British classification the onset is defined as “definite.” Both the CDC guidelines, lay emphasis on minor criteria, i.e. according to the CDC (1988) definition six or eight symptoms are required, although in the CDC (1994) definition, physical signs are dropped, the number of symptoms are decreased from eight to four and the total from eleven to eight symptoms. This will certainly increase the number of patients who fulfil the criteria and therefore this definition has to be regarded as less specific.

One of the major problems with all these case definitions, is that they rely on self-reporting and the reliability of this can only be improved with the introduction of a standardised fatigue scale.

COMPARISON OF THREE CASE DEFINITIONS OF CHRONIC FATIGUE SYNDROME

CRITERIA	CDC 1988	CDC 1994	BRITISH	AUSTRALIAN
Fatigue Duration	At least six months	At least six months	At least six months	At least six months
Fatigue Severity	50% decrease in activities	Substantial	Severe and disabling	Disruption in daily activities (substantial)
Other Characteristics of Fatigue	New onset	New onset	Definite onset	Postexertional fatigue
Neuropsychiatric Symptom	No requirements (maybe present)	No requirements (may be present)	Must affect mental functioning	Required
Other Diagnosis	Medical conditions associated with fatigue excluded	Medical conditions associated with fatigue excluded	Medical conditions associated with fatigue excluded	Medical conditions associated with fatigue excluded
Minor Criteria (symptomatic and physical)	Six or eight required	Four required	Not required	Not required
Psychiatric Exclusions	Psychosis, bipolar disorder, substance abuse	Melancholic depression, substance abuse, bipolar disorders, psychosis, eating disorder	Psychosis, bipolar disorder, eating disorder, organic brain disease	Psychosis, bipolar disorder, substance abuse, eating disorder

Table 1 - modified after Bates et al 1994 a.

Bates et al (1994 a) compared all the definitions and demonstrated that the percentage of the patients identified as having CFS were relatively similar when each of these definitions were used.

1.3 CRITERIA USED FOR DEFINING PATIENTS STUDIED IN THIS THESIS

Patients included in the studies of this thesis fulfilled the following criteria:

1. Acute onset of fatigue, which was severe enough to prevent them continuing in their occupation, prevented women from carrying out their household duties and dramatically altered their social and personal lives. It fluctuated with time and was made worse by exercise.
2. At least two of the following four were present:
 - a. Sleep disturbance (hypersomnia or insomnia).
 - b. Myalgia/arthralgia.
 - c. Neurobehavioural complaints (inability to concentrate, difficulty thinking, forgetfulness, anomia, atypical depression, generalised headaches).
 - d. Symptoms of irritable bowel syndrome - bloated stomach, colicky abdominal pain, alternating diarrhoea or constipation.
3. Patients with complaints of excessive sweating, chest pains, palpitations, dysequilibrium and recurrent infections were also included but this was not mandatory for inclusion into the studies.

4 Patients who were excluded from the studies included:

- a. Patients with established medical conditions known to cause chronic fatigue.
- b. Patients with a current diagnosis of schizophrenia, manic depressive psychosis or substance abuse.

In addition, all the patients included were seen at the Institute of Neurological Sciences and were hospitalized as inpatients. Detailed present and previous medical histories, a family, drug and occupational history were taken; physical and neurological examination and psychiatric evaluation were carried out. Laboratory tests included ESR, complete blood count and differential white blood count, protein electrophoresis, serum electrolytes, glucose, creatine, blood urea, serum calcium and phosphate, liver function tests, muscle enzymes (CPK and aldolase), urinalysis, chest x-rays, thyroid function tests, serum cortisol and anti-nuclear antibodies screen were also carried out in order to exclude any other medical cause for their fatigue.

1.4 HISTORY OF CHRONIC FATIGUE SYNDROME:

Chronic fatigue syndrome is not of recent origin. It has been described in the medical literature since the mid 1700s under a wide variety of names, each reflecting a particular concept of the syndrome's aetiology and epidemiology.

In 1750, Richard Manningham used the term "febricula" describing its features as including "little low, continued fever, ... little transient chilliness ... listlessness with great lassitude and weariness all over the body ... little flying pains ... sometimes the patient is a little delirious and forgetful" (Manningham 1750). He considered the

syndrome to be common in females, the sedentary and studious and to be precipitated by grief, intense thought and taking cold.

In the first half of the 19th century, Austin Flint used the term nervous exhaustion to describe chronic fatigue.

George Beard, an American neurologist, coined the term neurasthenia (Beard 1869), describing its features as profound fatiguability of body and mind. He considered it to be an organic illness: “it is a physical not a mental state” (Beard 1881) with increased prevalence in the educated and professional classes. Neurasthenia was defined as a condition of nervous exhaustion, characterized by undue fatigue on minimal exertion, both physical and mental, headache, gastrointestinal disturbances, and subjective sensations of all kinds (Cobb 1920) without any signs. Blocq (1894) reported that sufferers were well nourished, muscularly well developed and looked normal, although others described states of complete motor helplessness (Ferrier 1911). At the beginning of the 20th century these views were largely superseded by the new psychiatric diagnoses such as anxiety and depression, but traces of them survive in such conditions as chronic brucellosis, reactive hypoglycemia, chronic candidiasis and environmental hypersensitivity disorders. Neurasthenia itself remains a popular diagnosis in China, South East Asia and Eastern Europe.

Demise of Neurasthenia and Emergence of Post-infectious Fatigue Syndrome:

The first descriptions of neurasthenia included a link with febrile illness since Beard’s descriptions included “general and local chills and flashes of heat” (Beard 1880). This link was widely acknowledged by 1914 and various objective agents were held responsible for the illness (Robertson 1919; Craig 1922). These observations, however, were never confirmed scientifically and scepticism increased (Streckler 1920). The decline in neurasthenia did not end these attempts and in 1934, Alice

Evans tried to establish that chronic fatigue was the result of chronic brucellosis (Evans 1947) while chronic candidiasis was held responsible by Truss (1981) and Crook (1983). In the 1980's, several studies linked chronic fatiguability to the Epstein Barr virus. The term "chronic Epstein Barr infection" was introduced when some patients with chronic fatigue were found to have raised levels of antibodies to EBV antigens (Straus et al 1985; Straus 1988). Further investigations revealed that all these organisms can cause chronic illness, but such illnesses were readily distinguished from chronic fatigue syndrome, the term introduced in the USA (Holmes et al 1988) and Australia (Lloyd et al 1988).

A series of epidemics of CFS were reported between 1930 and 1960. These have been called after their location or by their resemblance to neurological conditions.

Epidemics:

1934 - Los Angeles City and California State, USA

The first epidemic occurred in the summer of 1934, a few weeks after an outbreak of poliomyelitis and affected 198 members of the medical and nursing staff of Los Angeles County General Hospital (Gilliam 1938). As the outbreak developed, certain clinical and epidemiologic features i.e. a high attack rate with a low mortality rate, a low paralytic rate and a high incidence in adults, appeared making the diagnosis of poliomyelitis unlikely. The epidemic ended, but over 300 sporadic cases were seen in southern California between 1948 and 1965 (Marinacci and Von Hagen 1965).

1937 - Erstfeld, Switzerland, Frohburg Hospital, Switzerland

In July 1937, out of 930 men stationed at Erstfeld, 130 were affected. No permanent disability was recorded (Stahel 1938). Later in the summer, another outbreak in St

Gall hospital at Frohburg was recorded, affecting patients and hospital staff. The features of the epidemic included meningeal involvement, muscle paresis, prolonged convalescence with relapses, autonomic disturbances and marked fatigability (Gsell 1938).

1939 - Degersheim, St Gallen, Switzerland

In September 1939, 73 out of 800 military officers who arrived at Degersheim from an area in which there was a poliomyelitis epidemic, developed symptoms suggestive of neuromyasthenia (Gsell 1949).

1942 & 1945 - Epidemic - Harefield Sanatorium, Middlesex, England & Pennsylvania, USA.

At the same time there was a small outbreak among young nurses at Harefield sanatorium, England (Houghton and Jones 1942). In 1945 an epidemic of pleurodynia with prominent neurologic symptoms and no demonstrable cause occurred in University Hospital of Pennsylvania, USA.

1948-1949 - Epidemic in Iceland

A large epidemic, originally diagnosed as poliomyelitis, broke out in the town of Akureyri, in Iceland, in the winter of 1948. Four hundred and sixty-five cases were reported (Sigurdsson et al 1950), mainly high school children in the age group of 15-19 years. The disease was characterized by mild fever, muscle tenderness confined to small spots, fatigability, disturbed sensations, emotional instability and irritability. Paresis of one or more muscles was present in 30% of the cases. The rapid spread of the epidemic in the town pointed to an infectious agent but all attempts at isolation were negative. The only clue as to a possible infectious agent was that 5 years later a large epidemic of poliomyelitis due to type 1 virus occurred in Iceland, affecting all

areas except those where Akureyri (Iceland) disease had occurred in 1948 (Sigurdsson et al 1958). Over 50% of the school children in the areas affected by poliomyelitis had antibodies to type 1 virus, but those in Akureyri had no specific antibody. In 1956 after polio vaccination, children in Akureyri produced unusually high specific antibody titres, suggesting that they had been exposed to an agent immunologically similar, but not identical to the polio virus.

1949-1951 - Epidemic in Adelaide, South Australia

In the Adelaide epidemic, the acute illness was short, diffuse muscle weakness was common, fever was trivial, recovery was rapid and there was no deaths (Pellew 1951). A recurrence of the disease, manifested as hyperacusis, depression and fatigue often occurred 4-8 weeks after the original illness.

1950 - Epidemic - New York State, USA

In the autumn of 1950 there was a widespread epidemic of poliomyelitis in New York State, USA. White and Burtch (1954) studied 19 cases and noted a close similarity to the description of the Icelandic outbreak. Lymphadenopathy, ulnar or sciatic nerve lesions, muscle tenderness and aching, paresis unaccompanied by a change in reflexes or muscle bulk, and depression were reported. It was concluded that the condition, though resembling it, was not poliomyelitis.

1952-1955

During these years different outbreaks were recorded from Middlesex Hospital, London (Acheson 1954), Whitley Hospital, Coventry and Coventry District, England (Macrae and Galpine 1954), Copenhagen, Denmark (Fog 1953), Lakeland, Florida, USA (Henderson and Shelokov 1959), Addington Hospital, Durban and Durban City, South Africa (Hill 1955), Dalston, Cumbria, England (Wallis 1955) and Royal Free Hospital, London (Compston 1956).

1955 - Epidemic - Royal Free Hospital, London, England

In the summer of 1955 a dramatic outbreak was recorded in the Royal Free Hospital. Out of 3,500 (staff, patients and medical students) some 300 experienced an illness described by Crowley and others (1957) as a lymphoreticular encephalomyelopathy. Instances of presumed case to case infection were recorded from the onset, with an incubation of 5 or 6 days. The earliest symptoms were of malaise, headaches, dizziness, myalgia and sore throat. More than half the cases showed tenderness and occasional enlargement of lymph nodes, liver and spleen; morphological abnormalities also occurred in the circulating lymphocytes consistent with those described in infective and allergic diseases. Neurological symptoms and signs developed in 74 % of cases and included hypersomnia, nightmares, panic states, labile mood and amnesia. Cranial nerve lesions, motor weakness and sensory disturbances were also recorded in a number of patients. Laboratory investigations failed to find any agent. The course of the disease was prolonged with relapses of varying intensity in most patients, although most cases had a complete physical recovery within 2 years.

Following the Royal Free outbreak, epidemics continued to be reported and their nature suggested that an infectious agent with an incubation period of 8-10 days must be responsible. In most cases an acute illness resulted, lasting for a few weeks, but in others the syndrome persisted for months or even years.

These epidemics pose many problems in their own right but are of undoubted historical relevance to the emergence of CFS as a disease. A contagious, paralytic illness with neurological signs, and a good prognosis appeared to be occurring. CFS in current medical practice, however, is non-contagious, fatiguing, without neurological signs and has a relatively poor prognosis.

1.5 GENERAL DATA

1.5.1 Prevalance

Data regarding the prevalence of CFS is variable because of the inconsistency in methodology as well as in case definitions in the relevant studies. Lloyd and colleagues (1990) in Australia recorded a point prevalence of 37 per 100,000 in a population based study using an operational definition. The CDC estimated the prevalence of CFS by surveillance of selected physicians in four US cities and gave lower figures than those in Australia, i.e. between 2 to 7 per 100,000 (Gunn et al 1993). In a primary care practice, Bates et al (1993) reported a point prevalence, according to the various definitions, of 0.3% (CDC 1988), 0.4% (UK) and 1% (Australian) respectively. Wessely et al reported a prevalence of CFS in the range of 0.8% (CDC 1988) to 1.8% (CDC 1994) across the south of England (Wessely 1995).

1.5.2 Geographical Distribution

The illness occurs both sporadically and in epidemics, with cases being reported from all over the United States, Alaska, Australia, Europe and South Africa (Acheson, 1959). Sporadic cases may continue to appear in the same geographic distribution after an epidemic has ended (Marinacci and Von Hagen 1965).

1.5.3 Gender

CFS has been documented in individuals of all ages, but young white females are at greater risk than others in the population (Parish 1978; Gunn et al 1993). In one community study, the relative risk of fatigue in women compared to men was 1.3 (Pawlikowska et al 1994), whereas in primary care the relative risk for women varied between 1.3 and 1.7 (Bates et al 1993).

There appears to be an unusual predisposition for persons in certain occupations to be affected, such as health service professionals (Parish 1978; Ramsey 1986) primary school teachers and farmers (Behan and Haniffah 1994).

1.5.4 Genetics

Whether or not genetics play any role in this illness is unknown but the possibility exists, as indicated by an increased history of an atopic diathesis in CFS patients, compared to controls (Olson et al 1986; Jones & Straus 1987). We have observed the illness in siblings, in father and son, or mother and daughter, and have collected a number of families in which related individuals lived a distance from each other but each developed CFS after a viral infection. We have also seen, albeit in small numbers, CFS in identical and dizygotic twins. In one instance, we saw the illness in three generations, each case having the illness precipitated by viruses and all those affected being females (POB - personal communication). These observations suggest a role for genetics in this illness.

Since patients with narcolepsy have a highly significant correlation with the possession of HLA-DR2 (Lock et al 1987), Behan and Behan (1988) looked at patients with CFS compared to controls: they found no difference in HLA-A, HLA-B and HLA-DR antigen frequencies. In contrast a limited number of studies have reported a higher than expected incidence of the HLA-D7 haplotype among patients with CFS (Tobi and Straus 1985).

1.6 AETIOLOGY

1.6.1 Infection

Chronic fatigue is a well-known clinical manifestation of systemic infections - caused by protozoa, fungi, rickettsia, spirochaetes, bacteria and viruses. The common protozoal

infections associated with fatigue are giardiasis, toxoplasmosis and trypanosomiasis or sleeping sickness. Trypanosomes are known to cause hypothalamic disturbances through the interaction of cytokines with hypothalamic receptors. The hypothalamic neurons which are involved in trypanosomal infection, express MHC Class 1 antigens early (Schultzberg et al 1989). These same neurones are involved in the production of neuroendocrine hormones including corticotropin releasing factor (CRF), angiotensin II, glucagon, oxytocin, vasopresin, cholecystokinin, dynorphin, LHRH, TRH and somatostatin (Armstrong 1985, Silverman and Pickard 1983). Disturbances in these substances may therefore explain, not only the symptoms of trypanosomiasis, but also of CFS. How fatigue is caused by other protozoal infections awaits elucidation. Among fungal infections, cryptococcosis and chronic candidiasis are associated with chronic fatigue.

In the early 1930s, enormous attention was given to bacterial infections as a cause of fatigue. Tuberculosis, a chronic bacterial infection, causes fatigue as one of its non-specific symptoms. Chronic brucellosis can be regarded as the prototype of bacterial infections which give rise to chronic fatigue. Dr Alice Evans, an American physician, in 1934 reported chronic fatigue as one possible manifestation of chronic brucellosis (Evans 1947). Later in the century, however, Spink and Imboden and their colleagues ended speculation about brucellosis as a direct cause of chronic fatigue in the absence of continued infection (Spink 1951, Imboden et al 1959.)

The link between CFS and viral infection was provided by epidemiological studies. The symptoms of fatigue during the first outbreak were similar to a mild form of poliomyelitis, and the disorder was originally described as “a disease resembling or simulating poliomyelitis” or “atypical poliomyelitis” (Acheson, 1959). Major epidemics of chronic fatigue were reported after or during outbreaks of poliomyelitis in Los

Angeles, Adelaide, and Durban (Gilliam 1938; Pellew 1951; Hill et al 1959). Similarly after the epidemic in Akureyri a widespread outbreak of poliomyelitis (Type 1 virus) occurred in the rest of Iceland, but interestingly none of the patients who had the fatigue syndrome were affected. Serologic studies showed that the patients were immune to poliomyelitis having already been exposed to a similar agent (Sigurdsson et al 1950; 1958).

From the analysis of such outbreaks Henderson and Shelokov were able to suggest that an infectious agent might have been involved, with an incubation period of 8-10 days and resulting in an acute febrile illness lasting up to 4 weeks (Henderson and Shelokov 1959). The evidence for viral infection is predominately anecdotal since it is a common observation that CFS may begin following a viral infection. The viruses implicated include enteroviruses, retroviruses and herpes viruses.

Enteroviruses

Behan et al reported increased neutralising antibody titres against Coxsackie B viruses in 70% of patients compared to 4% of healthy controls (Behan et al 1985). Other studies revealed that 82% of patients as opposed to 10% of control subjects had increased titres (Fegan et al 1983). The detection of enteroviral structural protein VPI in immune complexes from the serum of patients with CFS provided further evidence for a possible aetiological role of enteroviruses (Yousef et al 1988). Later studies, however, identified no differences in specific anticoxsackie antibody titres between patients and controls. Nonetheless, using molecular hybridisation techniques Gow et al (1991), Miller et al (1991) and Archard et al (1988) demonstrated the enteroviral sequences in muscle biopsies from patients with CFS. Cunningham et al (1990) reported that four out of eight (50%) patients' muscle biopsies were positive for enteroviral sequences and

Bowles et al (1993) found positivity in 26% of patients with CFS compared to 25.9% of patients with inflammatory myopathies.

Using the more sensitive PCR technology Gow et al (1991) reported that 53% of the CFS muscle samples were positive for enteroviral RNA sequences. Clements et al (1995) suggested that enterovirus was present in 42% of CFS serum samples. In contrast Swanink (1994) have shown there are no significant differences between patients and control samples, on serological and PCR testing.

These results do not exclude a role for enterovirus in initiating the disorder but do suggest that its pathogenesis is not dependent upon enteroviral persistence.

Retroviruses

DeFreitas and colleagues postulated a causal relationship between human T lymphotropic virus type II (HTLV-II) like retrovirus and CFS and found more than 75% patient samples were positive for this virus compared to 0% of non-exposure controls (DeFreitas et al 1991). Others in the UK (Gow et al 1992), USA (Khan et al 1993; Gunn 1993) and Japan (Honda et al 1993) have now reported negative results. Therefore it appears unlikely that HTLV-II like retrovirus is present in CFS.

Herpes Viruses

All the herpes viruses have been associated with CFS i.e., Epstein Barr virus, cytomegalovirus, varicella zoster virus, herpes viruses 6 and 7 and herpes simplex.

Epstein Barr Virus (EBV)

This group of herpes viruses has been studied extensively in relation to CFS. Several workers have reported that EBV infection may be followed by a chronic fatigue-like

syndrome, the "chronic mononucleosis syndrome" characterized by malaise, fatigue, low grade fever, loss of weight, and myalgia (Tobi et al 1982; Dubois 1984; Straus et al 1985). Significantly increased titres of antibodies to EBV-specific early antigen (EA) were reported in 20% of patients with CFS by Hotchin et al (1989). Similarly, 9% of CFS muscle samples were positive for EBV genome (Archard et al 1989). Despite these results, three seroepidemiologic surveys have indicated that EBV is unlikely to be the aetiologic agent (Hellinger et al 1988; Horwitz et al 1985; Lamy et al 1982) and others agree (Bell 1994).

Human Herpes Viruses 6 & 7 (HHV-6, HHV-7).

Buchwald and colleagues reported increased CD4/CD8 T cell ratios and replication of HHV-6 in the peripheral blood lymphocytes of 70% patients, as opposed to 20% of controls, in a well-controlled study of 259 cases at outbreaks in Lake Tahoe and California/Nevada (Buchwald et al 1992). Dale et al (1989) detected antibodies to HHV-6 in 69% of patients with CFS compared with 12.5% of controls. Wakefield et al (1988) however, found no difference in seroprevalence between patients and controls. Berneman et al (1992) isolated HHV-7 from the peripheral blood mononuclear cells of patients with CFS but subsequently, Secchiero et al (1994) isolated the same virus from a patient with CFS and a healthy blood donor. The significance of these findings, therefore, is still unknown.

The Concept of Viral Persistence in the Pathogenesis of CFS

One of the reasons for considering the herpes viruses as possible agents is that they are known to persist in latent or chronic forms after an initial infection.

In a chronic or persistent viral illness, the infected cells may not be killed and may reproduce, but are unlikely to carry out differentiated or specialised functions (Haywood 1986). For instance, neonatal mice infected with lymphocytic choriomeningitis virus (LCMV) show decreased production of neurotransmitters. Similarly, in neuroblastoma cells in culture infected with LCMV, although there is no cytopathic effect and cloning efficiency is normal, there is complete cessation of normal production of acetylcholine (Oldstone 1991). This idea, of persistent viral infection resulting in defective neurotransmitter production without evidence of cell damage, is important in understanding the pathogenesis of CFS. Recently McGarry et al (1994) detected enterovirus in the hypothalamus of a patient with CFS. Upregulation of hypothalamic 5HT receptors has been reported in CFS (Bakheit et al 1992), suggesting a link between persistent virus and decreased production of neurotransmitter (5HT), leading to the characteristic symptoms.

1.6.2 Metabolic

Several studies show that there is generalised disturbance of muscle metabolism in CFS. Early studies by Cohen et al (1947) at Harvard had shown abnormal responses to exercise: significantly greater rises in blood lactate were found after matched exercise protocols in patients with neurocirculatory asthenia, a disorder considered closely related to CFS (Straus 1991) compared to controls. This data was the first to question the idea that CFS might be a functional disorder and came down strongly on behalf of an organic aetiology. The work was repeated by Riley et al (1990) who also found abnormal lactate metabolism and reduced aerobic work capacity. An important criticism of both studies is the selection of controls, since the ideal control is a sedentary person who is unfit and neither study had such controls. What was also important in these early studies was that hyperventilation, which had been proposed as a cause of CFS, was shown to be completely untenable (Saisch et al 1994).

Arnold et al (1984) studied a case of CFS which followed chickenpox. Intracellular pH was measured using ^{31}P nuclear magnetic resonance spectroscopy (NMR) during ischaemic exercise. In comparison with healthy controls, the patient developed early and severe intracellular acidosis. This was presumed to be due to increased lactate formation as a result of excessive glycolytic activity, possibly caused by a change in the regulation of the relative contributions of aerobic and anaerobic metabolism to exercise. Subsequent studies by the same researchers on six other patients, showed similar abnormalities in four (Arnold 1984). These results have been confirmed by the comprehensive studies of Montago et al (1989) whose patients showed limited exercise capacity characterized by an inability to achieve the target heart rate, a lower exercise heart rate and reduced exercise duration on graded exercise testing. In addition to reduced duration of exercise Wong et al (1992) reported reduced intracellular concentration of ATP in their patients. This data suggests defective oxidative metabolism with a resultant acceleration of glycolysis in the working skeletal muscle. Finally, Lane et al (1995) using the subanaerobic threshold exercise test concluded that some patients with CFS have impaired muscle metabolism not explainable by physical inactivity or psychiatric disorder.

Defective oxidative metabolism suggests an abnormality at the cellular level probably involving mitochondria. In addition to nonspecific histological changes of mild to moderate atrophy of type 2 fibres, decreased numbers of type 1, occasional “moth-eaten” fibres, with increased lipid and/or glycogen deposition, Behan et al (1991) also reported distinctive changes in muscle mitochondria in patients with CFS: they were increased in number and size and showed an unusual pattern of branched cristae - called compartmentalisation.

Bryne et al (1985) looked at mitochondrial function in CFS by using polarographic techniques and found mild depression of state III respiration rates with site 1 and 2

substrates. In a later study, however, the same workers reported that skeletal muscle carnitine, phosphorylase, glycolytic enzymes and mitochondrial marker enzymes were normal (Byrne et al 1987). Wagenmakers and colleagues (1987) also reported impaired mitochondrial function but at the same time suggested that these defects could be entirely secondary to reduced muscle use and inactivity.

Japanese workers (Kuratsune et al 1994) and ourselves (Majeed et al 1995 a) have reported abnormalities of carnitine metabolism in patients with CFS. Carnitine has an important role in energy production and in modulation of the intramitochondrial co-enzyme A/acetyl coenzyme A ratio in skeletal muscle. Carnitine also plays an important part in muscle metabolism during exercise by controlling the influx of fatty acids into mitochondria. Oxidation of long chain fatty acids takes place within the mitochondria and is completely dependent on carnitine. It promotes the oxidation of pyruvate and branched amino acids and contributes to the protection of cells by preventing the accumulation of acetyl coA. Furthermore carnitine and carnitine acetyl-transferase are involved in the metabolism of acetyl coA and pyruvate oxidation (Rebouche et al 1983, Bernsen et al 1991).

Preedy et al (1993) have reported loss of muscle protein synthetic potential, without loss of muscle bulk. These findings may contribute to the perceived muscle weakness associated with CFS. In summary, the evidence for a metabolic abnormality in this condition is strong but precise explanation awaits elucidation.

1.6.3 Immunology

The clinical observations of the association of CFS with atopy, hayfever, asthma and other allergies (Straus et al 1988, Jones et al 1985), the development of hypersensitive/idiosyncratic reactions to certain drugs, recurrent infections, the symptomatology indistinguishable from that of CFS after administration of interferon to patients with chronic hepatitis B infection (McDonald et al 1987) may point to immune dysfunction in CFS. The concept of possible immune dysfunction in CFS and its relationship to post-viral states was first put forward by Behan et al in 1985 (Behan et al 1985). Since then different investigators have reported abnormalities in different elements of the immune system.

Serum immunoglobulin concentration results are conflicting. Most commonly immunoglobulin levels have been normal (Behan et al 1985, Borysiewicz et al 1986, Prieto et al 1989). Others have reported decreased amounts of immunoglobulins of the IgG, IgA, IgM or IgD classes (Lloyd et al 1989, DuBois et al 1984, Straus et al 1985, Salit 1985) while a recent report revealed increased IgG levels in 20% of patients compared to 0% of control subjects (Bates et al 1994). Deficiencies in IgG subclasses, IgG 1 or IgG 3 have been reported in 45% of patients studied (Komaroff et al 1988, Linde et al 1988, Lloyd et al 1989).

Several studies reported low levels of circulating immune complexes in CFS patients (Behan et al 1985, Borysiewicz et al 1986, Bates et al 1994) and decreased levels of complement are found in 0-25% (Behan et al 1985, DuBois et al 1984, Borysiewicz et al 1986) without any clinical manifestations of immune complex-mediated disease.

Autoantibodies:

Various circulating autoantibodies, in particular anti-nuclear antibodies and rheumatoid factor, are found in 20% and 10% of CFS patients respectively without other evidence of lupus or rheumatoid arthritis (Salit 1985, Tobi et al 1982, Jones 1985, Straus et al 1985).

Cytokines:

Cytokines (interferons) when administered to patients with chronic hepatitis B infections, resulted in symptoms described as “flu-like,” i.e., fatigue and myalgia, similar to those of CFS. On the basis of this observation it was postulated that the symptoms of CFS might result from the inappropriate production of cytokines. Later, different studies suggested that circulating interferons are infrequently, if ever, present (Borysiewicz et al 1986, Jones et al 1985, Straus et al 1985, Linde et al 1992). Interleukin 1 beta, interleukin 4, tumor necrosis factor and beta 2 microglobulin have not been detected in CFS. In contrast, higher levels of interleukin 2 (Cheney et al 1989), interleukin 6 and neuplerin (Chao et al 1990) have been reported in some patients with CFS, observations which have not been confirmed.

Lymphocyte Function:

Different studies report different findings in regard to lymphocyte phenotype and function. In general, the number of CD4 and CD8+ cells are normal (Behan et al 1985, Straus et al 1985, Borysiewicz et al 1986, Tirelli et al 1993), but increases and decreases have been reported (Behan et al 1985, Linde et al 1988). Buchwald and co-workers (1992) found a higher than normal CD4/CD8 ratio; whereas others demonstrated a normal (Landay et al 1991, Kilmas et al 1990) or decreased ratio (Hamblin et al 1983, DuBois et al 1984, Jones et al 1985, Linde et al 1988).

Natural Killer Cells:

Abnormalities of natural killer (NK) cell number and functions have been reported in a number of studies. A reduction in the absolute number and percentage of NK cells has been seen from 0% to 73% of patients (Behan et al 1985, Caligiuri et al 1987). NK cell function has been found to be increased (Gold et al 1990), decreased (Caligiuri et al 1987, Kilmas et al 1990) or normal (Borysiewicz et al 1986), as measured by cytolytic activity against a number of different target cell lines.

Overall such immunological studies suggest a host response to an infection that may be persistent or may have been eliminated but has led to the activation of the immune system. No single immunological finding has been identified as typical of CFS.

1.6.4 Psychiatry:

Two important points which suggested that CFS might be purely a psychiatric disorder were, first, fatigue is a major symptom of the clinical spectrum of several different psychiatric illnesses and secondly, physical and laboratory abnormalities could not be detected.

Different studies have confirmed that fatigue is the commonest somatic symptom of an affective disorder, occurring in the majority of patients (Mathew et al 1981, Wittenborn and Buhler 1979, Hamilton 1989). Beck (1973) reported increased fatigability in 62% of mild, 80% of moderate and 86% of severely depressed patients, compared with 40% of controls. On the other hand, 80% of fatigued patients in primary care had depression and/or somatic anxiety (Kroenke et al 1988). In one study, Research Diagnostic Criteria for a major depressive disorder were satisfied in 47% of a hospital sample of CFS patients, after removing complicating somatic symptoms from the diagnostic criteria

(Wessley and Powell 1989). Despite this association, the response to anti-depressant medication has been poor in a number of cases of CFS.

Procter (1991) explained the association between CFS and depression on the basis of a depression vulnerability hypothesis because of the increased prevalence of lifetime psychiatric disorders in the CFS group (Taerk 1987), a depression - reactivity hypothesis because of the reaction to a chronic state of ill health, the loss of role functioning and the degree of uncertainty about the diagnosis and prognosis of the disorder (Ramsay 1986, Jones and Miller 1987) and an organic hypothesis because of changes in neurotransmitter neuroendocrine functions as a result of disease processes (Bakheit et al 1992, Majeed et al 1995 b). Finally she suggested in her study that CFS depression has much in common with a reactive disorder secondary to medical illness.

Due to overlap between the symptoms of CFS and neurotic depression, the separation of these two entities is sometimes difficult, if not impossible. They share certain symptoms and have some common neuroendocrine abnormalities. Nonetheless CFS is now being recognised as a separate entity and a psychiatric aetiology would be untenable.

1.6.5 Autonomic Nervous System:

The symptoms of palpitation, diarrhoea, urinary frequency/nocturia suggest involvement of autonomic nervous system in CFS. Studies showing abnormal heart rate responses to exercise in CFS also suggest autonomic dysfunction. Rosenhall et al (1987 a & b) reported abnormal auditory brain stem responses in patients with chronic fibromyalgia/CFS and pointed out that autonomic dysfunction in these patients is secondary to brain dysfunction. They also documented abnormal eye movements in these patients and suggested that brain dysfunction was at the brain stem level. The dysequilibrium which is present in 30-50% of patients with CFS could be a manifestation

of a vestibular or central nervous system disorder (Furman 1991). In order to resolve the issue of peripheral versus central CNS involvement, Ash Bernal et al (1995) tested vestibular function in eleven patients and found that abnormalities were more suggestive of central nervous system deficits than of peripheral vestibular dysfunction.

Sisto et al (1995) have found recently that patients breathing at specific rates, have diminished vagal activity in both the sitting and standing postures, compared to healthy controls. These abnormalities in vagal function again suggest autonomic brain dysfunction.

1.7 CLINICAL FEATURES

1.7.1 Mode of Presentation

The majority of patients have an acute onset with a 'flu-like illness, accompanied by upper respiratory symptoms or gastro-enteritis (Acheson 1959. Holt 1965). The illness may occur after encephalitis, labyrinthitis, myocarditis, orchitis or prostatitis (Behan et al 1988). These modes of presentation clearly implicate an infectious agent at the onset. Another common mode of presentation is with a syndrome akin to Bornholm disease, with anterior chest pain and myocarditis. As the disease continues, myalgia becomes generalised and disproportionate fatigue appears. Another presentation is with acute vertigo, which lasts for about a week and then leaves the patient with a constant feeling of unsteadiness or dysequilibrium, severe exhaustion and varying degrees of myalgia.

These presentations are not mutually exclusive and can occur in any combination.

1.7.2 Precipitating Factors

There is evidence that infection plays a role in precipitating the illness. Many previous epidemics have been described and more than 80 % of the patients ascribe their illness to an infectious event. Indeed, the infectious element is highlighted by the other names of the illness, i.e. acute infective encephalomyelitis, atypical poliomyelitis, a disease resembling or simulating poliomyelitis, persistent myalgia following a sore throat and chronic infectious mononucleosis syndrome (Behan et al 1988). What is now clear is that infection, usually of a mild type, can precipitate the illness, but there is no direct evidence for persisting viruses causing the continuing disease. Enteroviruses, influenza, varicella-zoster, Epstein Barr virus and hepatitis virus, have all been implicated as precipitating agents. The illness has also been observed in farmers and agricultural workers exposed to sheep dips and insecticides containing organophosphates (Behan et al 1994). These patients often claim to have a 'flu-like illness before developing CFS. Patients may also develop CFS after gastro-enteritis due to ciguatera poisoning (Gillespie et al 1986).

1.7.3 Symptoms

Fatigue

Human fatigue is a baffling symptom which has escaped accurate definition and measurement. Bartley describes the symptom in his book 'Fatigue, Mechanism and Management' (Bartley 1965) as a "sensory, cognitive syndrome which includes tiredness, aversion to work, body discomfort, ineffectiveness in performance ... a self-felt assessment of inadequacy ... with the desire to escape."

In neurophysiological terms fatigue can be defined as failure to maintain a required or expected force (Edward 1986). Muscle fatigue can be further categorised as "central" where there is volitional or non-volitional failure of neural drive to the muscles or

“peripheral” where there is failure in force generation by mechanisms at or beyond the neuromuscular junction. In neuropsychology fatigue can refer to time related decrements in the ability to perform mental tasks and varies according to personality and social desirability (May and Kline 1988).

Fatigue as a Manifestation of Psychiatric Disorder

The great majority of patients who enter a hospital because of unexplained chronic fatigue and lassitude have been found to have some type of psychiatric illness. Chen (1986) looked at the risk of anxiety and depression in a community sample of fatigued patients; 46% of males and 51.1% of females, were depressed. In a hospital-based study however, 67% of 135 self-referrals to a special fatigue clinic were found to have psychiatric diagnoses (Marn et al 1988). Wessley and Powell (1989) studied 47 medical referrals to a specialist neurology hospital with chronic unexplained fatigue, and found that 72% had a psychiatric diagnosis.

Fatigue in Chronic Infections

Infection is another cause of chronic fatigue, though a much less frequent one. Infection should be suspected when the fatigue is out of proportion to other symptoms such as mood change, nervousness and anxiety, e.g. in hepatitis, tuberculosis, brucellosis or infectious mononucleosis.

Fatigue in Metabolic and Endocrine Diseases

Metabolic and endocrine diseases of various types may cause inordinate degrees of lassitude and fatigue. In Addison’s and Simmond’s diseases, fatigue may dominate the clinical picture. Aldosterone deficiency is another established cause of fatigue. In persons with hypothyroidism, lassitude and sluggishness are frequent complaints.

Uncontrolled diabetes mellitus may be accompanied by excessive fatigability, as may hyperparathyroidism, hypogonadism, and Cushing's disease. Anemia when moderate or severe should be considered as a possible cause of unexplained fatigue. Among neurologic diseases; Parkinson's disease and multiple sclerosis, are ones in which fatigability is a prominent symptom and they should also be considered.

Fatigue in CFS

Fatigue in CFS varies from day to day and after exercise. Patients will claim that they are weary, have no energy and are unable to complete tasks that they could do formerly without any problem. The typical fatigue is noticed within the first few days after the precipitating event, i.e. infection or toxin exposure. It fluctuates, being made worse by muscle exercise, mental tasks or emotional stress. Further infections often aggravate the fatigue.

Some patients are able to carry out their normal physical activity, although feeling fatigued but in other patients, the fatigue progresses to such an extent that they are unable to care for themselves and stay in bed, needing day to day care and assistance. It is still not clear whether the fatigue experienced by CFS patients is peripheral in origin, or is a consequence of an alteration in central drive. Gibson et al (1993) studied the physiological function of a large muscle group before, during and after exercising activity in a well-defined population of patients with CFS and found normal contractile function. The longterm recovery of muscle was identical to that of normal subjects and complete by 24 hours after exhaustive exercise, even though the patients complained of excessive fatigue, weakness or muscular discomfort. Differences in the perception of effort in relation to the physiological response (raised perceived exertion scores) to exercise, indicated the likely involvement of central mechanisms in limiting exercise performance.

As Denman states “our understanding of chronic fatigue has reached an important stage and progress will only be achieved if different categories of chronic fatigue are dissected with scientific objectivity and therapeutic reason” (Denman 1990).

Conditions with Central Fatigue

Multiple Sclerosis

It has long been recognised that fatigue may be a central incapacitating symptom in patients with multiple sclerosis. In their classical treatise on multiple sclerosis, which has not been bettered, MacAlpine, Lumsden and Acheson point out that “fatigue usually arises from periods of hyperactivity but routine work and daily journey induce it, particularly in those whose general health is of low standard (MacAlpine et al 1972).

We have observed in young people in whom multiple sclerosis has been precipitated by whiplash injury, that severe disabling fatigue was the main symptom, associated with hypersomnia.

Post Head Injury Depression

Depression following minor closed head injury is a common phenomenon shown to be different clinically, in its response to treatment and in its neuroendocrine responses, to idiopathic depression (Dinan & Mobayed 1992).

The patients often have severe fatigue with increased somnolence as part of their symptom complex. Brainstem auditory-evoked responses have been reported as abnormal and detailed neuroendocrine responses measuring prolactin release or using the dexamethasone suppression test suggest a different response to that seen in idiopathic depression (Saran 1985). Saran found that few patients with depression after mild head injury were non-suppressors on the dexamethasone suppression test in

contrast to the functional depressives. Clearly, the atypical depression that follows closed mild head injury bears a striking resemblance to, and should be considered in the differential diagnosis of, chronic fatigue syndrome.

Post-Polio Syndrome

Post-polio syndrome is a recently described disorder in which muscle weakness associated with a variety of other symptoms occurs in patients who have had acute poliomyelitis previously (Dalakas et al 1986). The new development of weakness occurs many years after apparent recovery from acute paralytic poliomyelitis. Fatigue is the most commonly reported, most debilitating sequel affecting more than 1.63 million American polio survivors, with 91% reporting new or increased fatigue, 41% reporting fatigue significantly interfering with performing or completing work and 25% reporting fatigue interfering with selfcare activities (Parsons 1989, Bruno 1987).

Polio survivors differentiate between the physical tiredness and decreased endurance they associate with new muscle weakness and a “brain fatigue” that is characterized by problems with attention and cognition similar to those in patients with CFS. Neurophysiological, muscle biopsy, and cerebro-spinal fluid findings differentiate these patients from those with CFS but the clinical symptomatology is virtually identical to it.

Idiopathic Cyclic Oedema

This is a well recognised condition in women who experience fluid retention in the absence of known causes such as hypoproteinaemia, renal, hepatic, alimentary or cardiac disease. The condition overlaps with the normal pre-menstrual tension and weight gain at the time of the menses. An abnormal amount of fluid is retained, affecting the face, arms and abdomen, and sometimes producing headache. The fluid retention can occur throughout the day and as much as four to five pounds can be gained between morning and evening and throughout the menstrual period when, at times, there can be as much as twelve to fourteen pounds in weight gain. At the time of maximum weight gain, the patients complain bitterly of incapacitating fatigue associated with difficulty in concentration and memory. The condition is exacerbated by stress and particularly by infections. Several of the patients that we have seen bore a striking clinical similarity to patients with CFS, heightened in the subgroup who also went on to develop diabetes mellitus.

The pathophysiology of this condition is unknown but there is evidence for a hypothalamic disturbance (Young et al 1983). Attention has been drawn in the past to the other features of this syndrome, in particular the depression, emotional lability and overwhelming chronic fatigue that may be experienced. Several patients studied by us had undisputed CFS. The finding of increased water content in these cases and in patients with CFS suggests an overlap between the two entities (Bakheit et al 1993). The underlying mechanisms appear to be aggravated by female sex hormones.

Myalgia

Diffuse muscle pain involving large muscles such as the trapezii, muscles of the shoulder, chest, quadriceps and gastrocnemii often accompany infections such as dengue, brucellosis, influenza, measles, malaria, salmonellosis and toxoplasmosis, trichinosis, Weil's disease and, of course, rheumatic fever. Severe pain associated with coxsackie enteroviral infections and involving the chest muscles, has long been known as Bornholm disease. Patients with idiopathic polyneuritis may have pain in their legs at the onset of the disorder, as can patients with acute poliomyelitis. Shingles is a recognised form of segmental muscle pain. In essence, therefore, acute viral, fungal, bacterial infections can all be associated with pain in muscles, but the mechanism is not understood. Inflammation may result in the release of a number of pain producing substances, but it is noteworthy that in polymyositis, pain is usually absent, unless tendinous structures are involved. Muscle pain in polymyalgia rheumatica is another poorly understood syndrome, as is fibromyalgia which is a condition identical with CFS. Pain is common in the metabolic myopathies, and may be precipitated and made worse by exercise as in CFS. In CFS, the pain is usually generalised, tends to affect the larger muscles and is characteristically fluctuating and made worse by exercise. In some patients it is mild but in others, it is the overwhelming symptom. On examination, it may be associated with tenderness, particularly at certain points in the muscle. The cause of the pain is unknown, but present data suggest a metabolic type of induced myalgia (Behan et al 1991).

Neuropsychiatric Symptoms

Neuropsychiatric symptoms in chronic fatigue syndrome have received a lot of attention, but few objective studies carried out, (Smith et al 1993). Patients complain of disturbances of memory and concentration, of emotional lability and of irritability, of

anomia and of a reluctance to take in and carry out mental tasks. There was no difference between healthy controls and patients with chronic fatigue syndrome of the free recall tasks, but the patients were more cautious in the delayed recognition tasks, i.e. they made fewer false alarms, but detected fewer target words. This study showed that the performance impairment in these patients could not be the result of depression alone (Smith et al 1993).

About 80% of patients have psychiatric symptoms, but it is important to note that 20% are entirely free of any history of, or present, psychiatric complaints. Phenomenological differences have been found in presenting psychiatric and attributional style, which distinguish chronic fatigue syndrome patients from psychiatric populations. In one study, (Powell et al 1990), chronic fatigue syndrome subjects satisfying criteria for depression showed depressed mood and emotional lability, often with profound pessimism about the future, but did not display active suicidal ideation, feelings of guilt or negative self referential cognitions (low self-esteem, self-depreciation), which were familiar among a group of psychiatric inpatients with major depression. The chronic fatigue syndrome subjects demonstrated significantly less loss of pleasure in comparison to the depressed group and attributed their illness almost exclusively to a physical (viral) cause, (Wessely & Powell 1989). An extraordinary feature which they do show is hypergraphia: the patient will bring to the clinic long descriptions of his or her symptoms, a phenomenon described by the late Professor Norman Geschwind in patients with temporal lobe epilepsy (Geschwind 1979). Patients often complain of difficulty in memory but this is more forgetfulness than a true loss of memory. They often have conspicuous anomia and it is this anomia, a common feature in virtually all encephalopathic processes, that they misinterpret as memory disturbances.

Sleep Disturbance

The majority of the patients sleep excessively at the beginning of the illness. The hypersomnolence is a conspicuous feature and patients will complain that they can drop off to sleep at the slightest opportunity. Even after as much as 12-14 hours of deep sleep, however, they awake unrefreshed. With the passage of time, however, patients often develop difficulty in getting off to sleep and wake early. Whelton et al (1992) showed that CFS patients had more difficulty falling asleep and spent less time asleep. They had reduced rapid eye movement (REM) sleep and more alpha EEG in non-rapid eye movement (NREM) sleep than healthy controlled subjects, a sleep anomaly which is considered to be an indication of a physiologic arousal disorder and is accompanied by indicators of increased vigilance during sleep and the subjective experience of unrefreshing sleep (Anch 1991).

Irritable Bowel Syndrome

In our experience, the irritable bowel syndrome which occurs in CFS (though not a part of CFS definition) is indistinguishable from the idiopathically occurring irritable bowel syndrome. Patients complain of intractable constipation, alternating with diarrhoea or abdominal pain and loose stools throughout the day. Interestingly, we found few patients with nocturnal diarrhoea, but most patients complained of bloating, palpitations, irregularities of bowel motility and dyspepsia. The mechanisms underlying irritable bowel syndrome are unknown, but there is some experimental data to implicate 5-HT.

Dysequilibrium

A significant number of cases of CFS begin with sudden vertigo and dysequilibrium persists in 30-50% of cases. Several patients have had abnormal neuroaudiological findings suggestive of brain stem dysfunction (Rosenhall et al 1987). A previous study by Furman (1991) indicated that the dysequilibrium seen in these patients could be a

manifestation of vestibular or central nervous system disorder. A central nervous system deficit rather than peripheral vestibular dysfunction was postulated (Ash Bernal et al 1995). Epidemics of CFS with vertigo have been described in the past. Pederson found “intense asthenia which develops in five to ten per cent of the cases and often becomes the predominant symptom in the late course.” A significant number of his patients at the beginning had a pleocytosis in the cerebrospinal fluid and he considered the illness to be an infection of the brain stem (Pederson 1959).

Atopic Disorders

In our experience, large numbers of patients have a strong atopic diathesis, suffering from asthma or hayfever and other allergies. Strauss in a study of 27 patients who were entering a placebo control trial of Acyclovir for CFS found that, “there was a remarkable correlation between patient history and skin test reactivity. Of 12 reactive patients, 10 had histories of allergies to food or inhalants, of 12 non-reacting patients, only 3 reported allergies to food or inhalants ($P < 0.01$), however, even among individuals who did not identify themselves allergic to food or inhalants there was evidence of atopy by history. In all 11 of 12 skin test reactors and 9 of the 12 non-reactors provided information suggestive of atopy, such as eczema, seasonal rhinitis, hives, asthma and drug or food allergies.” (Straus et al 1988). While the nature of this association is unknown, there is little doubt that atopic allergies have some connection with CFS.

Night Sweats

Patients often complain of extreme degrees of sweating, particularly at night, although they may occur at any time during the day. The sweating is pathological in that they report not only having to change their pyjamas, but often having to change the sheets as well. The night sweats, the hot and cold sensations and the sudden changes in appetite, probably reflect subtle hypothalamic disturbances.

1.8 EVIDENCE FOR HYPOTHALAMIC INVOLVEMENT

The clinical and laboratory data which suggests involvement of central (hypothalamic) mechanisms in the pathophysiology of CFS are now discussed.

1.8.1 Clinical Data

The following clinical features of this illness are suggestive of involvement of hypothalamus. Some patients with CFS develop a fluid retention syndrome (Behan and Bakheit, 1991), in which fatigue may be a prominent feature (Pelosi 1986). Although the pathophysiology of this syndrome is unknown, disturbance at hypothalamic level has been reported (Young et al 1983). Indeed, German and Stempfer (1979) have suggested that fatigue itself is primarily an exhaustion or malfunctioning of brain cells in the hypothalamic region, since they observed a good response to hypothalamic-releasing factor in reactive depression where prolonged stress and exhaustion had played a role. In panhypopituitarism after most hormones have been replaced it is known that the patient still complains of fatigue and this has been shown to be due to abnormalities of either prolactin or growth hormone (Martin 1987), again suggesting involvement of hypothalamus. Other complaints of night sweats, feelings of hot and cold and of a raised temperature, may also reflect a hypothalamic disorder (Nikitopoulou and Crammer 1976). In view of the psychological and emotional problems, sleep disturbances and mood changes that accompany CFS, and the fact that major depressive disorders are often associated with a disturbance of hypothalamic pituitary axis (Ettigi and Brown 1977), it was thought that hypothalamic functions might be impaired in this illness also. Finally, patients with CFS often complain of an exaggeration in their symptoms, especially fatigue, at the time of menstruation (Deisher 1957), emphasising the hormonal influence on the clinical features of this illness.

1.8.2 Laboratory Evidence

The following laboratory investigations on patients with CFS suggest involvement of hypothalamus.

Impaired HPA-axis activation.

Reduced basal evening cortisol, low 24 hour urinary-free cortisol excretion and elevated ACTH concentration have been reported in CFS. Enhanced adrenocortical sensitivity to exogenous ACTH and blunted ACTH responses to CRH have been found, indicating central adrenal insufficiency secondary to either a deficiency of CRH or to some other central stimulus for the pituitary adrenal axis (Demitrack et al 1991). Demitrack and co-workers used corticotropin-releasing hormone (CRH) to stimulate ACTH release from the anterior pituitary. Tests were conducted at 2000h. A significant reduction in baseline cortisol levels and an elevation in baseline ACTH was noted. Overall the ACTH release in response to CRH was blunted. An examination of CRH and ACTH levels in cerebrospinal fluid revealed no abnormality. A series of tests using various dosage of ACTH to stimulate cortisol release from the adrenals was carried out. The lowest dose of ACTH produced a marked elevation in cortisol in patients with CFS, but no elevation in healthy controls. With higher doses patients with CFS showed attenuated responses, it was suggested on the basis of these results, that patients with CFS have a form of mild hypocortisolism, reflecting a deficit at the level of the hypothalamus or above.

Disturbed Water Metabolism.

Bakheit et al (1993) studied water metabolism and the response of the neurohypophysis to changes in plasma osmolality during the water loading and water deprivation tests, in patients with CFS reported that secretion of arginine-vasopressin (AVP) was erratic, as shown by lack of correlation between serum and urine osmolality and the corresponding plasma AVP levels. Patients with CFS also had significantly lower baseline arginine-vasopressin levels compared with healthy subjects. It was suggested that the results were indicative of hypothalamic dysfunction.

Disturbed 5HT/serotonin function.

- a. Prolactin responses to the 5HT_{1A} receptor agonist were investigated by comparing prolactin release with buspirone in patients with CFS to that with depression and healthy controls (Bakheit et al 1992). There was no significant difference between patients with chronic fatigue and healthy subjects or depressives, in terms of baseline prolactin concentration. The percentage difference between peak and baseline prolactin values was significantly higher in patients with CFS than in the controls or the depressives. The results were however interpreted as having supersensitivity of 5HT_{1A} receptors in CFS.
- b. Cleare et al (1995) measured serum prolactin and cortisol concentrations hourly, following oral administration of the selective 5HT releasing agent d-fenfluramine in patients with CFS, depression and healthy controls and found cortisol levels were highest in the depressed and lowest in CFS. Peak cortisol responses did not differ between the groups. Basal prolactin values were similar in the three groups while peak prolactin responses were lowest in the depressed and highest in the patients

with CFS. They concluded that defective prolactin release in CFS was due to increased 5HT function in hypothalamus.

McGarry et al (1994) detected enteroviral sequences in brain samples from the hypothalamus and brain stem region of a patient with CFS which may be significant.

GENERAL NEUROENDOCRINOLOGICAL DATA

2.1 INTRODUCTION

The endocrine and nervous systems regulate almost all the metabolic and homeostatic activities of the organism. They interact and influence each other. The rate of most endocrine secretion is influenced directly or indirectly by the brain transmitters, and virtually all hormones can influence brain activity. The basic functional unit of the endocrine system is the secretory cell, releasing substances into the blood in which they are carried via the circulation to selective target tissues where they modify activity. The basic functional unit of the nervous system, the neuron, exists in anatomically distinct pathways and mediates its regulatory functions via electrical impulses. Nerve cells and endocrine cells however have many attributes in common. Neurons, in common with endocrine cells, secrete chemical messengers that react with specific receptors and both have electric potentials. Several kinds of peptides and neurotransmitters which are synthesized by nerve cells are identical to those secreted by endocrine glands (Roth et al 1982). Neuroendocrinology looks at the interface between the endocrine and nervous systems in an attempt to understand their complex relationship.

Aspects of this interface are examined in the studies of this thesis i.e. the effects of various neurotransmitters on the hypothalamic pituitary adrenal axis (HPA) in patients with CFS.

2.1.1 Hormones

A hormone is a chemical substance that is secreted into the body fluids by one cell or a group of cells and exerts a physiological effect on other cells of the body. All hormones share several characteristics. First, they are present in the circulation at low concentration. Second, they are directed to sites of action by specific mechanisms. This direction is accomplished by specific receptors with high affinity in target tissues, recognising and binding the hormone. Another mechanism by which hormones can be

directed to specific target tissues is by delivery within a restricted circulation such as the hypophyseal-portal system or the hepatic-portal system. A third means of targetting is by direct diffusion to adjacent sites. A fourth mechanism is local formation of hormone within a tissue from circulating precursors as with the formation of dihydrotestosterone from testosterone within androgen target tissues such as prostate (Wilson and Foster 1985).

Third, hormone synthesis is regulated in general by feedback control mechanism (Burgi 1974). The regulation is accomplished by hormones produced peripherally in endocrine glands “feeding back” on the hypothalamic pituitary system: the concentration of the hormone signals the need for more or less production.

Fourth, rhythms in the release of hormones are a common feature of almost all endocrine systems. These rhythms can vary over minutes to hours (pulsatile secretion of prolactin [PRL] luteinizing hormone [LH] and testosterone), days (the circadian variability in cortisol secretion), weeks (the menstrual cycle), or even longer periods (seasonal variability in thyroxine production).

2.1.2 Chemical Nature of Hormones

Hormones fall into two main categories - peptides and steroids. The majority are polypeptides and range from very complex polypeptides such as luteinizing hormone [LH], through intermediate sized peptides (somatostatin), to small peptides (thyrotropin releasing hormone [TRH]) and dipeptides (thyroxin).

2.1.2.1 Peptide Hormones

Hormones of hypothalamus: - [corticotropin releasing hormone (CRH), thyrotropin releasing hormone (TRH), gonadotropin releasing hormone (GnRH), growth hormone releasing hormone (GHRH), somatostatin], anterior pituitary [growth hormone (GH), adrenocorticotropin hormone (ACTH), thyroid stimulating hormone (TSH), follicle stimulating hormone (FSH), luteinizing hormone (LH), prolactin (PRL) and melanocyte stimulating hormone], posterior pituitary [antidiuretic hormone (vasopressin - AVP) and oxytocin] and of the pancreas - [insulin and glucagon], thyroid - [thyroxin, triiodothyroxin and calcitonin] and adrenal - medulla [catecholamines] - are peptide hormones. These water soluble hormones do not cross the lipid barrier interposed by the plasma membrane but interact directly with receptors located on the cell surface. The receptors are specific with high affinity for the corresponding hormone; binding of which results in activation of the second messenger system in the plasma membrane - 3', 5'-adenosine monophosphate (cyclic AMP) and inositol triphosphate and diacylglycerol.

Cyclic AMP System

Activation of adenylate cyclase, catalytic unit (C), catalyzes the conversion of adenosine triphosphate (ATP) to cyclic AMP. Cyclic AMP activates protein kinase A which phosphorylates proteins producing physiologic effects. Stimulatory ligands bind to the stimulatory receptor (Rs) and activate adenylate cyclase via Gs - the stimulatory

nucleotide regulatory protein. Inhibitory ligands inhibit adenylate cyclase via the inhibitory receptor (R_i) and inhibitory nucleotide regulatory protein (G_i). See Figure (2.1) for diagrammatic representation of this process.

Inositol Triphosphate and Diacylglycerol System

Other peptide hormones when coupled with target receptors R activate phospholipase C (PLC) on the inner surface of the membrane via nucleotide regulatory protein (G). The resulting hydrolysis of phosphatidylinositol 4,5 diphosphate (PIP₂) produces inositol 1,4,5 triphosphate (IP₃) which releases calcium from endoplasmic reticulum (ER) and diacylglycerol (DAG) which activates protein kinase C (PKC). Protein kinases are intracellular enzymes that phosphorylate proteins implicated in the production of many different cellular responses (Berridge 1984).

2.1.2.2 Steroid Hormones

The steroid hormones are produced by the adrenal glands, the ovaries and the testes and all share a characteristic chemical structure. They are large molecules derived from the cholesterol and have a basic steroid “nucleus” of 17 carbon atoms joined together in four interconnected rings. The major groups of steroids are glucocorticoids (cortisol and corticosterone), the mineralocorticoids (aldosterone) produced by the cortex of the adrenal gland and sex steroids (testosterone, oestradiol and progesterone) secreted by the testes and ovaries. The chemical structure of the steroid hormones is illustrated in Figure(2.2).

The steroid hormones are lipid soluble and traverse the lipid rich membranes of the targets cells mostly by diffusion. In the target cells steroid binds to macromolecules called receptors. These molecules are relatively large proteins that have specific binding

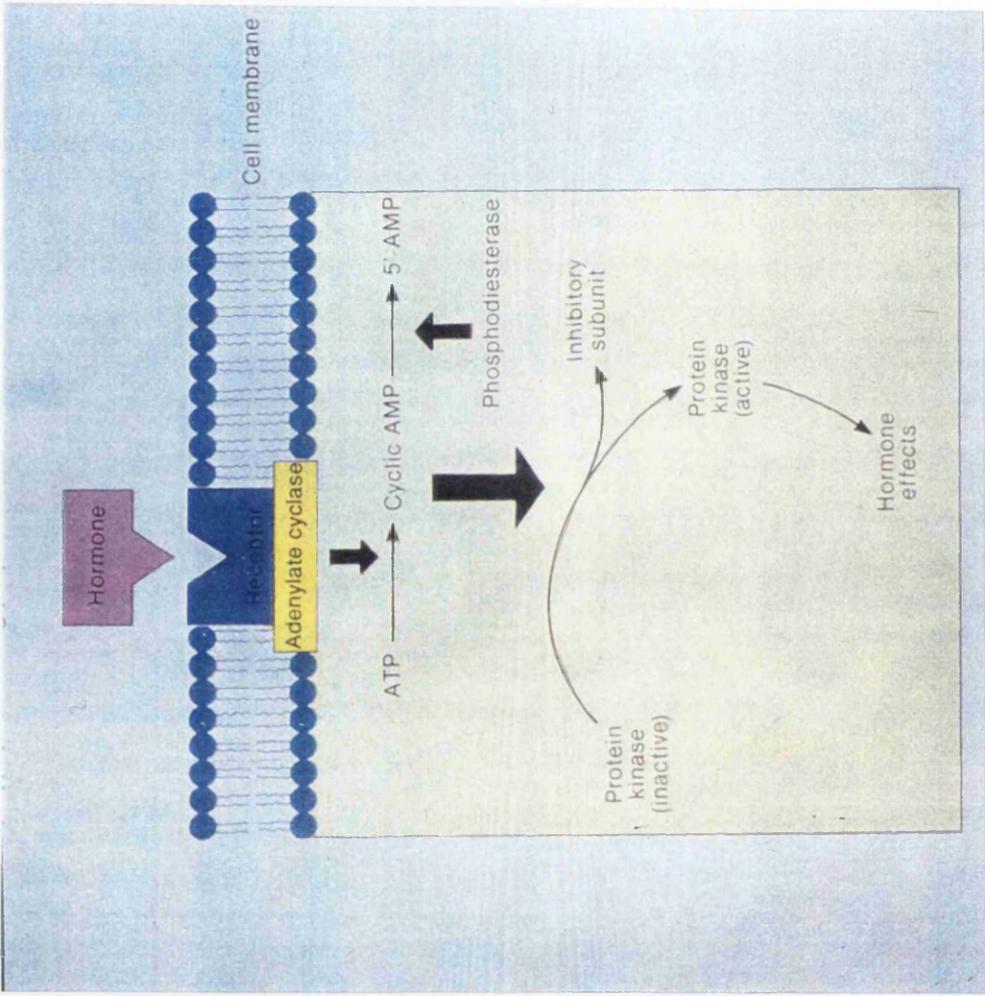
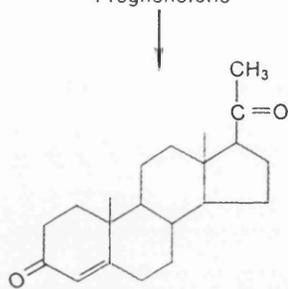
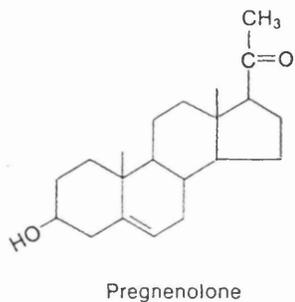
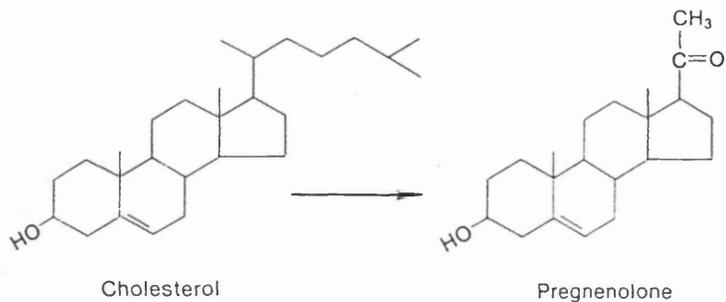
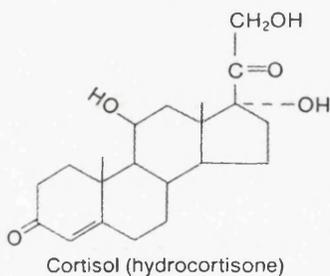


Figure 2.1

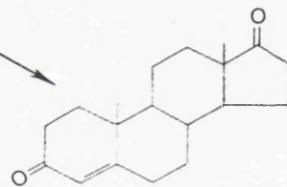
Cyclic AMP (cAMP) as a second messenger in the action of polypeptide and glycoprotein hormones.



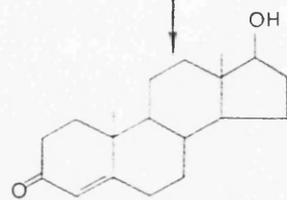
Secreted by ovaries



Secreted by adrenal cortex

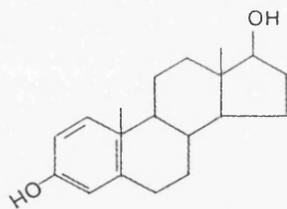


Androstenedione



Testosterone

Secreted by testes



Estradiol - 17 β

Secreted by ovaries

Figure 2.2

Biosynthetic pathways for steroid hormones and their chemical structures.

sites for the hormone and are found in both the cytoplasmic and nuclear fractions of the cell. The delivery of steroid hormone to receptor is heavily dependent on the concentration of carrier proteins: proteins that bind steroids and transport them in the circulation to target tissue. Once in the cell and bound to receptor, the hormone receptor complex translocates to the nucleus and binds to DNA (Rousseau 1973), activating transcription of target genes. The messenger RNA (mRNA) is translated into new proteins that express the biological activity of the glucocorticoid. See Figure (2.3). Steroid hormones also have membrane receptors and can act directly upon the cell to produce an immediate effect such as altering the firing rate of the neurone.

Steroid Hormone (Glucocorticoid) Receptors

Two types of central adrenal steroid receptor have been described - first observed by McEwen et al (1968) after they injected a tracer dose (0.5-1 microgr) of [H] corticosterone into adrenalectomized (ADX) rats.

Type 1 are mineralocorticoid receptors (MR-receptors); and Type 2 are glucocorticoid receptors (GR-receptors). The type 1 receptors have tenfold higher affinity for corticosterone than do glucocorticoid receptors in the rat brain (Reul et al 1985) and are found in high concentrations in extrahypothalamic limbic neurons of the septo-hippocampal complex, whereas in the pituitary, relatively low levels are found (Reul and Dekloet 1986). They also have a high affinity for aldosterone and have been described by Beaumont and Fanestil (1983) as mineralocorticoid-like receptors.

Type 2 (GR-receptors) resemble physiochemically the classical GR system in the periphery and have the highest affinity for synthetic glucocorticoids such as dexamethasone with a lower affinity for binding naturally occurring glucocorticoids (Reul and Dekloet, 1986; Sutanto and Dekloet, 1987). They are found in both neuronal and glial cells. They are present in high quantities throughout the forebrain, particularly in

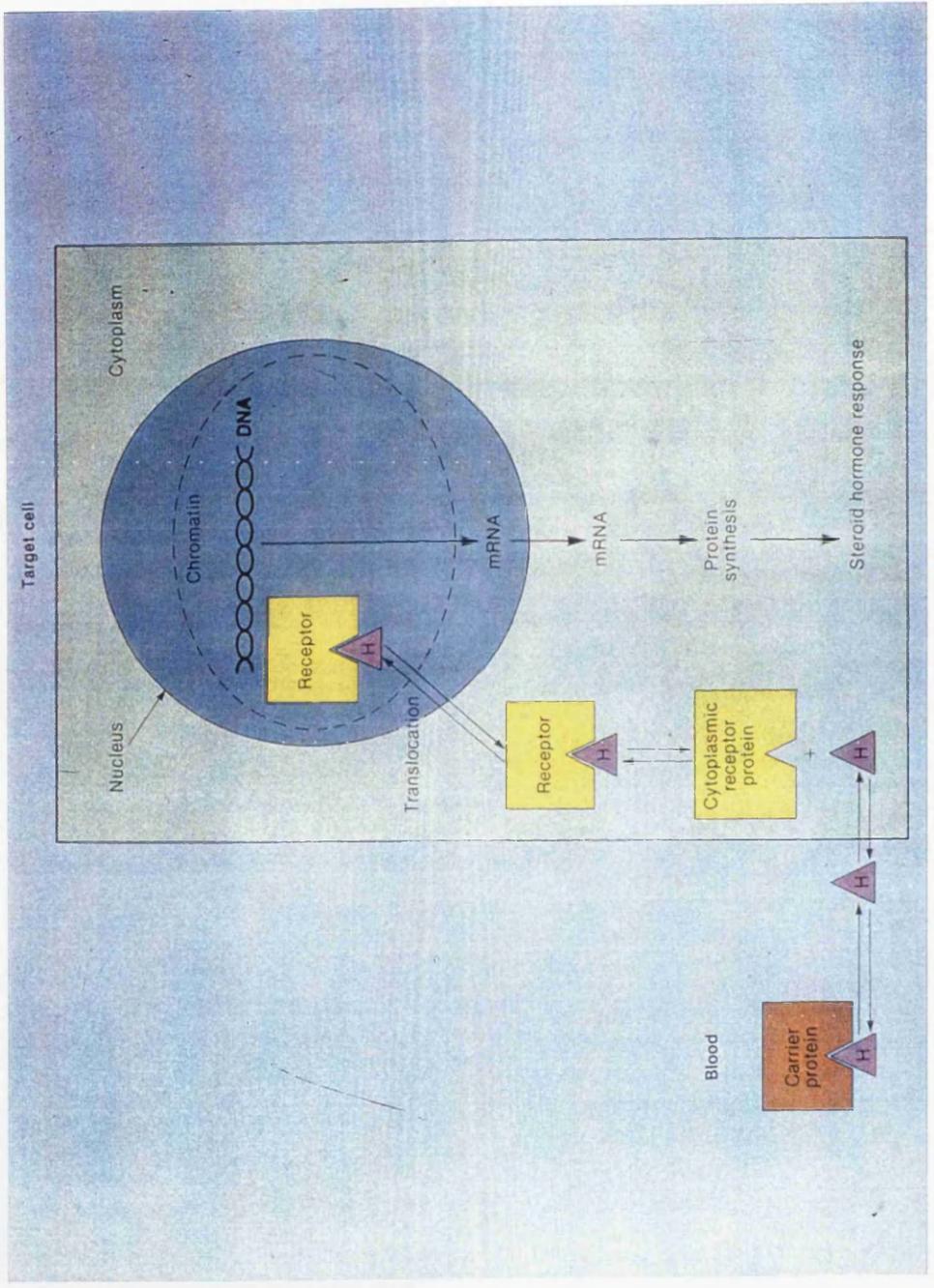


Figure 2.3

The mechanism of action of a steroid hormone (H) on the target cells.

cortical, thalamic, hypothalamic (paraventricular, arcuate, supraoptic nucleus) and septohippocampal (dorso-lateral septum, dentate gyrus) regions. (Reul and Dekloet, 1986). In immunocytochemical studies using a monoclonal antibody against rat liver glucocorticoid receptor, Van Eckelen et al (1987) found strong reactivity in CA1 and CA2 pyramidal cells in the hippocampus. They found that the activity was highest in the parvocellular neurones of the paraventricular nucleus of the hypothalamus and in the tractus solitarius and nucleus locus coeruleus in brainstem nuclei.

More recently Reul et al (1989) used ³²p-labelled cRNA probes directed against type 1 and type 2 receptor messenger RNA to examine the distribution of these receptors in the rat. Among the nervous tissues, mRNA coding for mineralocorticoid receptors was highest in the hippocampus. Moderate to low levels were found in the hypothalamus and cerebellum respectively. In situ hybridization histochemistry revealed that in the brain mineralocorticoid receptors appear to be expressed exclusively in neurons (Arriza et al, 1988 and Van Eckelene et al 1988). Heavy labelling occurred in the pyramidal neurons of Ammon's horn with the CA3 cell field exhibiting the highest mineralocorticoid receptor mRNA levels. The distribution of GR mRNA was rather evenly distributed over the brain. Nevertheless, hypothalamus tissue contain more GR mRNA than did hippocampus and cerebellum. In situ hybridization showed glucocorticoid receptor mRNA within the hippocampus to be primarily present in the neurons of the CA1 and CA2 field (Aronsson et al 1988). Very low concentration of glucocorticoid receptors mRNA were found in the CA3 and CA4 cell field. Apart from the hippocampal formation, increased labelling of glucocorticoid receptor mRNA was present over the granular layer of the cerebellum, in the subdivision of the amygdala, and in the arcuate nucleus and parvocellular region of the parventricular nucleus of the hypothalamus (Aronsson et al 1988). Further profound labelling was found over the aminergic cell groups of the locus coeruleus and raphe nuclei. Moderate glucocorticoid

receptor mRNA concentrations were present in well defined layers of the cortex, central nucleus of the amygdala, thalamic regions, and most of the preoptic and hypothalamic nuclei. Glucocorticoid receptor mRNA was low in the caudate nucleus, putamen, septor region, and substantia nigra.

Studies involving measurement of glucocorticoid receptor mRNA and binding sites have revealed that glucocorticoid receptors are subject to autoregulation. After adrenalectomy (ADX), glucocorticoid receptor concentration increases but it is reduced after chronic stress, chronic administration of glucocorticoids, and at senescence. A diminished glucocorticoid receptor concentration may compromise the negative feedback action exerted by glucocorticoid after stress. After ADX, mineralocorticoid receptor binding is upregulated acutely and reaches its maximum between 7 and 24 hours post-ADX. Long-term ADX has no effect on the mineralocorticoid receptor concentration, but, interestingly chronic dexamethasone treatment results in an up-regulation of mineralocorticoid receptors. Functionally, mineralocorticoid receptors and glucocorticoid receptors are involved in different aspects of the organisation of the stress response and in conjunction they control the response to stress of the animal.

2.2 HYPOTHALAMIC-HYPOPHYSEAL AXIS

The classical endocrine system is regulated by the hypophysis which in turn is regulated by the neurosecretory cells of the hypothalamus. These neurosecretory neurons, in turn, are regulated by neurotransmitters whose cell bodies originate both within and without the hypothalamus. A brief outline of the anatomy and physiology of the structure is necessary to understand the outline and aims of the studies that follow.

2.2.1 The Hypothalamus

The hypothalamus is situated at the base of the diencephalon, beneath the thalamus and on each side of the third ventricle. The basal surface is defined by the optic chiasm anteriorly and the mamillary bodies posteriorly. In between is a grey swelling called the tuber cinereum which tapers ventrally into the infundibulum which, together with the infundibular part of the adenohypophysis, forms the hypophyseal stalk. This infundibular region at the base of the hypothalamus is frequently called the median eminence. It is the site at which hypothalamic neurones that regulate the anterior pituitary release their secretions in an anatomic relation to the primary plexus of the hypophyseal-portal system. This region is also the site through which neurones that end in neural lobe and intermediate lobe of the pituitary pass (Flerko 1980, Lechan 1987). Three components of this structure can be identified: neural, consisting of nerve terminals and neurones in passage; vascular, consisting of the primary capillary plexus and portal veins and epithelial, consisting of the pars tuberalis of the anterior pituitary gland. This primary capillary plexus formed by the hypophyseal branch of the internal carotid artery drains into portal vessels which pass into the adenohypophysis. These vessels are the conduit through which the secretions of the tuberoinfundibular neurones reach the pituitary (Page 1983). There the portal vessels break up to form a second capillary bed which bathes the endocrine cells and drains finally into the cavernous sinus.

It is customary to divide the hypothalamus into three regions: anterior (supraoptic), middle (tuberal) and posterior (mamillary). The preoptic, supraoptic, superchiasmatic, paraventricular and anterior nuclei are located in the anterior region. The middle region contains the arcuate, tuberal, lateral, dorsal, dorsomedial, ventromedial, posterior and periventricular nuclei. The mamillary and posterior nuclei are situated in the posterior region (see Figure 2.4).

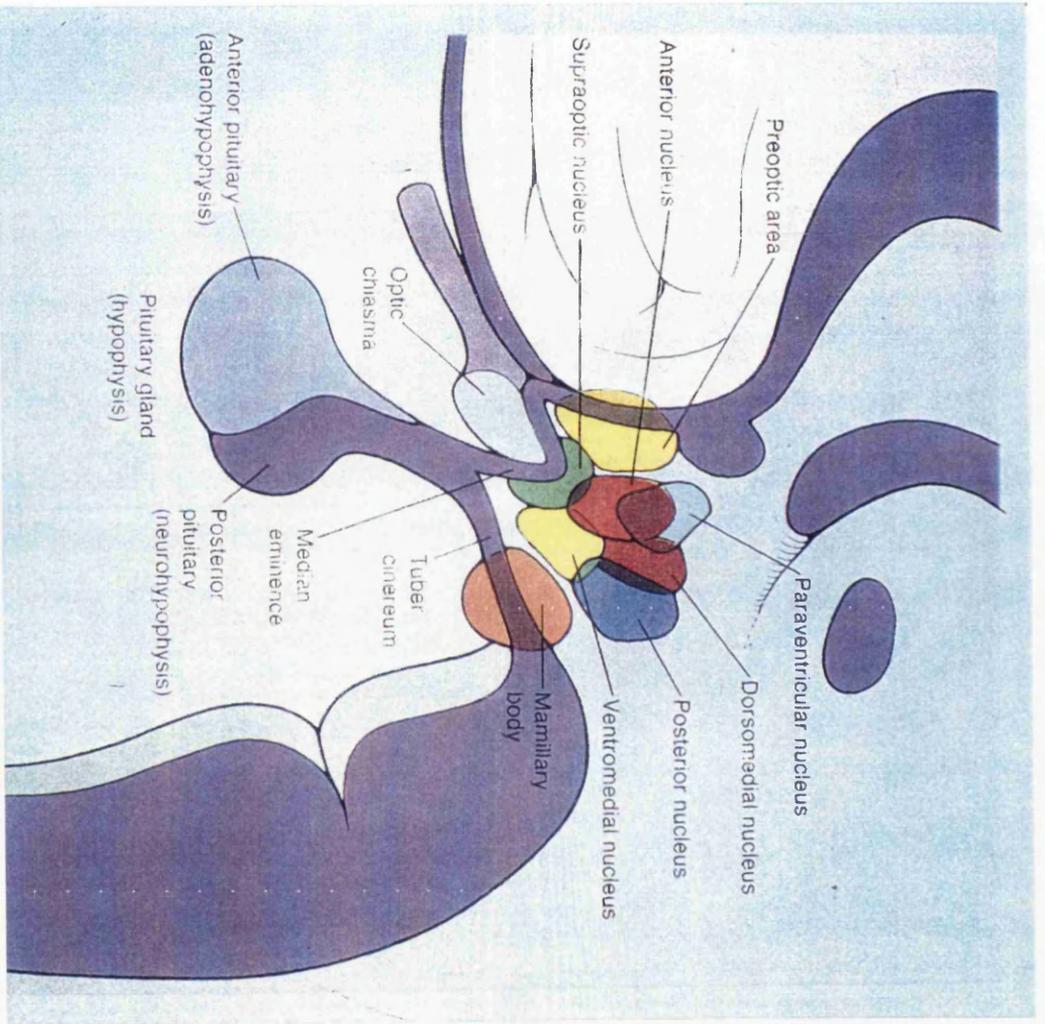


Figure 2.4

Diagram of the nuclei within the hypothalamus.

Hypophyseotropic Hormones of the Hypothalamus

The currently used term, releasing factor, is applied to hypothalamic substances of unknown chemical nature, whereas substances with established chemical identity are referred to as releasing hormone. The cells of each releasing hormone have distinct distributions but they all converge on the median eminence where they come into contact with the capillaries of the hypophyseoportal plexus. The cells projecting to the median eminence have been demonstrated by retrograde transport methods (Lechan, 1982) and it is within the cell bodies of these neurons that the hypothalamic neurohormones are synthesised.

The chemical structures of all of the classic releasing factors have now been established. All (thyrotropin releasing hormone [TRH], gonadotropin releasing hormone [GnRH], somatostatin, growth hormone releasing hormone [GHRH] and corticotropin releasing hormone [CRH]) are peptides with one important exception, dopamine, which is the principal prolactin release inhibitory factor.

Certain hypothalamic factors exert significant inhibitory actions on anterior pituitary function. Inhibitory factors interact with the respective releasing factor to exert dual control of the secretion of prolactin (PRL) growth hormone (GH) and thyroid stimulating hormone (TSH). Further the actions of hypophyseotropic hormones are not limited strictly to a single pituitary hormone; for example thyroid releasing hormone (TRH) is a potent releaser of prolactin (PRL) as well as of thyroid stimulating hormone (TSH) and, under certain circumstances, may release ACTH and GH (Borges et al 1983). LHRH releases both LH and FSH. Somatostatin inhibits the secretion of GH, TSH and a wide variety of other non-pituitary hormones. The principal inhibitor of PRL secretion, dopamine, also inhibits TSH, gonadotropin and, under certain conditions, GH secretion.

Secretion of releasing hormone is regulated by local neurotransmitters and neuropeptides which interact with the effects of circulating hormone such as glucocorticoid, gonadal steroids and thyroid hormones. There is also evidence for feedback effects of anterior pituitary hormone (short loop feedback control) and of hypophyseotropic factors themselves (ultrashort loop feedback control).

Many of these hypophyseotropic factors are distributed and sometimes synthesized in sites beyond the hypothalamus or the CNS. There are extensive aggregations of hypothalamic CRH cell bodies and terminal CRH neurones in the limbic system, cortex, striatal and hippocampal areas and in close association with the locus coeruleus. TRH has been identified by immunoassay or immunohistochemistry in virtually all parts of the brain including the cerebral cortex, basal ganglia, neurohypophysis and spinal cord; as well as in pancreatic islet cells and various parts of the gastrointestinal tract (Engler et al 1981; Jackson 1982). GnRH is found outside the hypothalamus in a number of regions of the limbic system (Hsueh and Jones 1981). GHRH is not present in the normal gut or pancreas and within the CNS is largely limited to the tuberoinfundibular nervous system. Somatostatin is the most widely distributed of the hypothalamic peptides within both the nervous system and many extra neural tissues, including the gut and pancreas.

2.2.2 The pituitary

Anatomy

The pituitary is located in the sella turcica or hypophyseal fossa of the skull, under the brain (Goddyer 1989). It is protected by the sphenoid bone, which surrounds it bilaterally and inferiorly, and enclosed in the dura that lines the sella turcica. Superiorly it is covered by the diaphragma sellae, a dural sheath that forms the roof of the sella. The diaphragma sellae has a central opening that is penetrated by the hypophyseal stalk. The pituitary is an oval, bean shaped, bi-laterally symmetrical, brownish-red organ. The

weight of the pituitary varies in adults, averaging 0.6 gm and weighing somewhat more in women than in men. During pregnancy it enlarges and may reach 0.9-1.0 gm (Scheithauer 1990). The anterior lobe is larger than the posterior lobe and constitutes 80% of the gland. The anterior lobe is composed of three divisions: pars distalis, pars intermedia, and pars tuberalis (Goodyer 1989). The pars distalis is the largest and is the site of the hormone-producing cells. The pars intermedia is poorly developed in the human, consisting of a few dilated cavities filled with an amorphous proteinaceous material. The pars tuberalis is the upward extension of the anterior lobe, attached to the pituitary stalk. It contains a few small groups of cells including squamous cells and produces mainly glycoproteins. The structure of the pituitary gland is shown in Figure 2.5.

The neurohypophysis or posterior pituitary consist of three parts (Sheehan and Kovacs 1982): the median eminence of the tuber cinereum, the infundibular stem or hypophyseal stalk, and the posterior lobe or neural lobe. The dominant features of the neurohypophysis are the large cell tracts (hence called magnocellular) which originate in the supraoptic and paraventricular nuclei, descend through the infundibulum and the neural stalk and terminate in dilated endings in the neural lobe (as shown in Figure 2.6). The principal biologically active substances secreted here are vasopressin (anti-diuretic hormone) and oxytocin. Anterior pituitary hormones are listed in Table (2). The studies of this thesis measure some hormones from the anterior pituitary; namely GH and prolactin and the adrenal steroid cortisol, whose release is stimulated by the anterior pituitary ACTH. A brief account of the regulation of the hormones is given below.

Regulation of Growth Hormone

Human growth hormone is a non-glycosylated, single chain, 191 amino acid, 22 kd protein with two intramolecular disulfide bonds. Approximately 75% of the pituitary

The structure of the pituitary gland as seen in sagittal view.

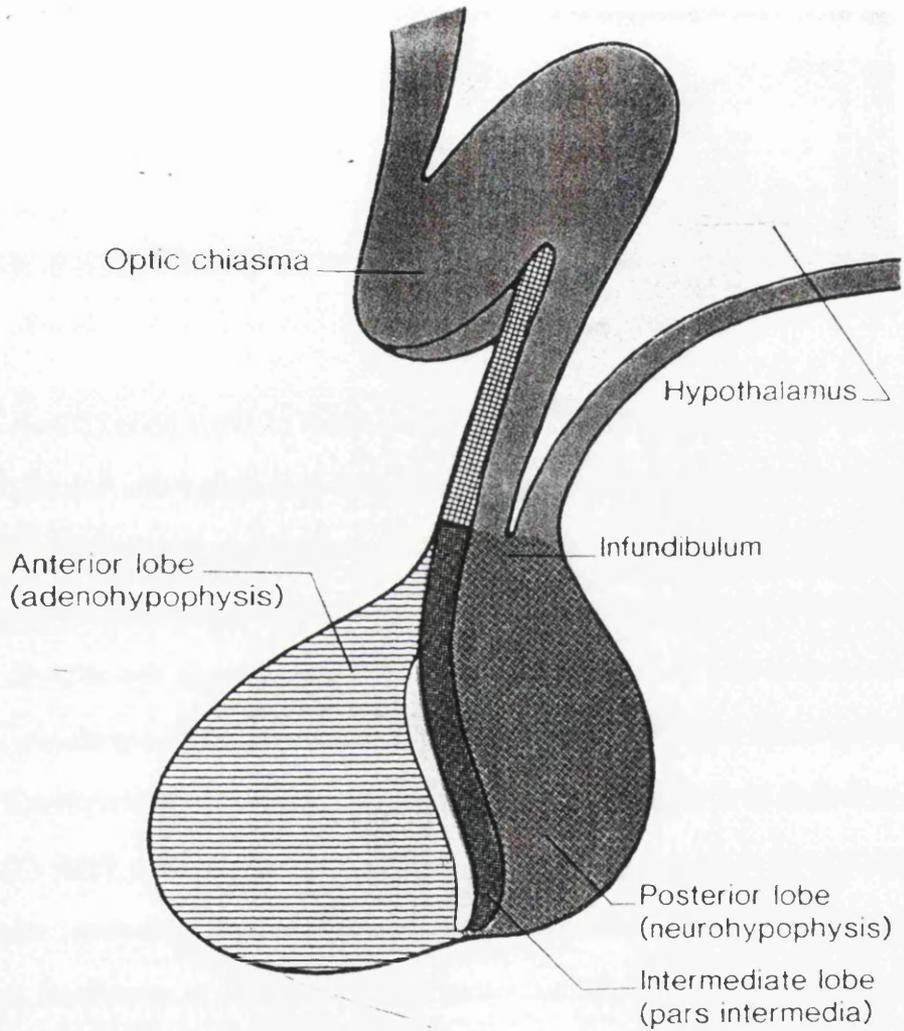


Figure 2.5

The structure of the pituitary gland as seen in sagittal view.

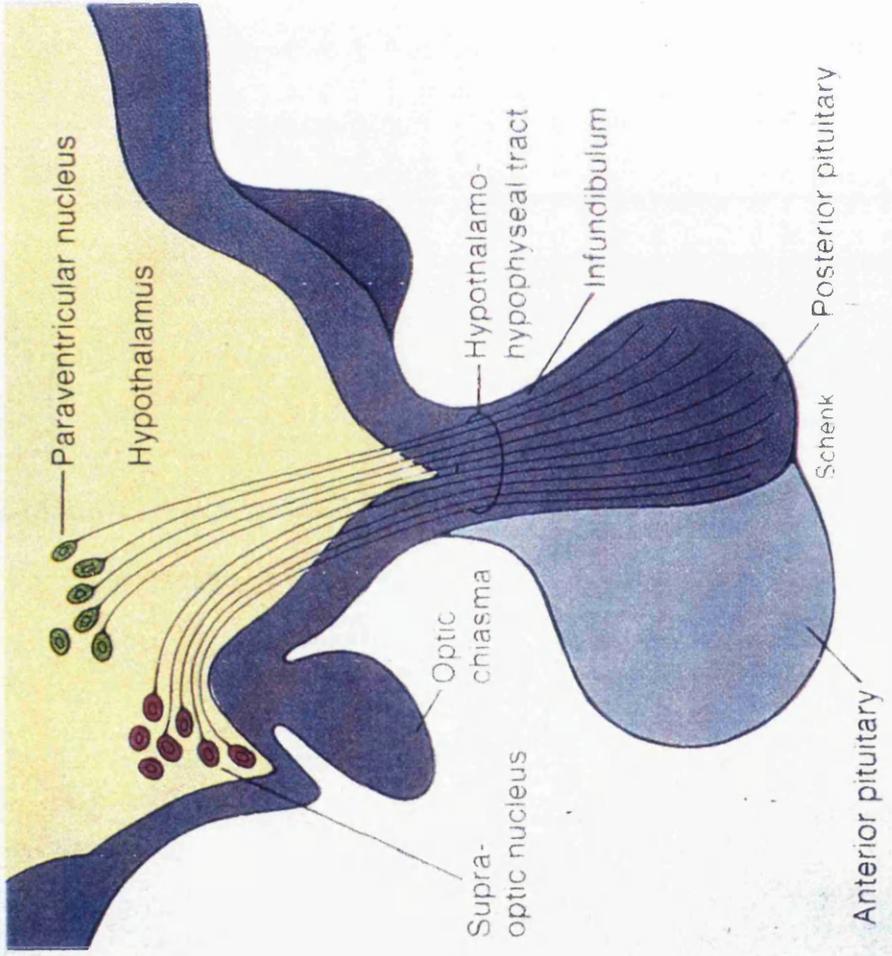


Figure 2.6

The posterior pituitary or neurohypophysis, stores and secretes hormones (vasopressin and oxytocin) produced in neuron cell bodies within the supraoptic and paraventricular nuclei of the hypothalamus. These hormones are transported to the posterior pituitary by nerve fibres of the hypothalamo-hypophyseal tract.

TABLE 2**Anterior pituitary hormones and their regulation.**

HORMONE	TARGET TISSUE	STIMULATED BY HORMONE	REGULATION OF SECRETION
ACTH (adrenocorticotrophic hormone)	Adrenal cortex	Secretion of glucocorticoids	Stimulated by CRH; inhibited by glucocorticoids.
TSH (thyroid stimulating hormone)	Thyroid gland	Secretion of thyroid hormones	Stimulated by TRH; inhibited by thyroid hormones.
GH (growth hormone)	Most tissue	Protein synthesis and growth; lipolysis and increased blood glucose.	Inhibited by somatostatin; stimulated by growth hormone - releasing hormone.
FSH (follicle-stimulating hormone) and LH (luteinizing hormone)	Gonads	Gamete production and sex steroid hormone secretion.	Stimulated by GnRH; inhibited by sex steroids.
Prolactin	Mammary glands and other sex accessory organs	Milk production	Inhibited by PIH (prolactin-inhibiting hormone).

GH is in this form and 5-10% is in a 20 kd form. The somatotrope cells that secrete GH make up about 50% of the hormone producing cells of the anterior pituitary and occupy the lateral wings of the pituitary. The human adenohypophysis contains 5-10 mg of GH, synthesised and stored in the somatotropes. The gene for human GH is on chromosome 17 (Owerbach 1980).

The pulsatile secretion of GH is regulated by two hypothalamic regulatory hormones - GHRH and somatostatin (SRIF, somatotropin release - inhibiting factor), as shown in Figure 2.7. GHRH controls GH synthesis by regulating transcription of GH mRNA via control of cAMP levels (Barinaga 1983). Somatostatin appears to determine the timing and amplitude of GH pulses but has no effect on GH synthesis. In addition to the hypothalamic influences, the somatotrope is regulated by negative feedback by circulating somatomedins such as insulin-like growth factor (IGF, also called somatomedin - C) at the pituitary and hypothalamic levels and by short loop feedback by GH itself on the hypothalamus. The pattern of GH secretion depends upon a number of factors including stage of development, nutritional state, sleep stage, stress and exercise. Exercise, stress and some neurogenic factors stimulate GH secretion. Central alpha-adrenergic agonists (norepinephrine) stimulate GH secretion; clonidine, a central alpha-adrenergic agonist, stimulates GH secretion in this manner. Beta-adrenergic antagonists augment the efficacy of various stimuli for GH secretion. Dopamine agonists stimulate GH secretion in normal subjects by stimulating dopamine receptors. Acetylcholine agonists stimulate and agents that lower acetylcholine tone suppress GH release.

Growth Hormone Releasing Hormone

This polypeptide was sequenced (Rivier et al; 1982 and Guillemin et al 1982) using extracts of human pancreatic tumours which had caused acromegaly. Three molecular forms of human GHRH exist: a 40 amino acid linear peptide with a free carboxyl

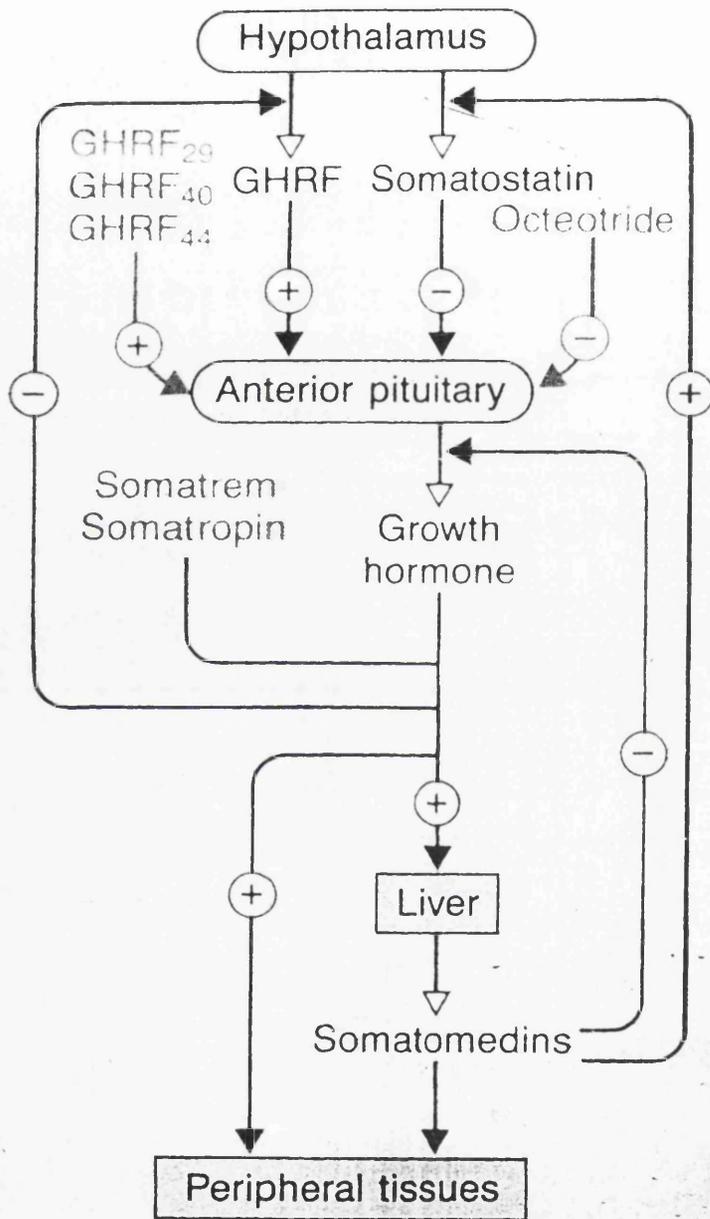


Figure 2.7

Control of growth hormone secretion and its actions. The diagram displays analogues of growth hormone and growth hormone-releasing factor (GHRF). Synthetic peptides with GHRF activity consist of 1-29, 1-40 and 1-44 amino acid residues.

terminal (GHRH 1-40 OH), a 44 amino acid sequence with an amidated carboxyl terminal (GHRH 1-44 NH₂) and a 37 amino acid peptide (GHRH 1-37-OH). GHRH is cleaved from a larger precursor molecule of approximately 13,000 Daltons (preprosomatocrinin) (Mayo et al 1983). GHRH-containing cell bodies are largely confined to the arcuate and to a lesser extent, the ventromedial nuclei of the hypothalamus. Bundles of axons project down from these cells to end in close proximity with the portal vessels in the posterior area of the median eminence.

Somatostatin

Somatostatin was first characterised as a hypothalamic tetradecapeptide (SS-14) which inhibited the secretion of GH. It is cleaved from a precursor molecule, a 116 amino acid peptide called preprosometostatin (Shen et al 1982). The highest concentration of SS in the nervous system is found in nerve terminals adjacent to the primary capillaries of the hypophyseal portal blood vessels in the median eminence (Krisch 1978; Johansson et al 1984). These nerve terminals have their origin in a concentrated group of SS producing cell bodies which lie close to the third ventricle (periventricular nucleus) and extend from the preoptic area to the rostral pole of the median eminence. SS-containing cell bodies are abundant in a number of other hypothalamic nuclei, notably the arcuate, ventromedial and supra-chiasmatic nuclei. There is good evidence indicating that the actions of somatostatin on the pituitary are mediated by binding to a single population of high affinity somatostatin receptors. Somatostatin produces cAMP accumulation in anterior pituitary tissue which is paralleled by progressive decreases in GH and TRH release. This suggests that somatostatin may exert inhibitory effects by blocking cAMP formation. There is substantial evidence that neurosecretory SS neurons in the hypothalamus receive a catecholaminergic (probably dopaminergic) input (Arimura and Fishback 1981). Thus intraventricular dopamine stimulates SS secretion and inhibits the release of GH. However, circulating GH levels were increased following peripheral

administration of L-DOPA or dopaminergic agonists. This paradoxical effect suggests that both SS and GHRH may be regulated by dopaminergic stimuli, and that the effects of dopaminergic agents on pituitary GH secretion may reflect a delicate balance between the effects of the release of these two regulatory factors. GH can exert negative feedback on its own secretion both directly, by stimulating the release of SS from the hypothalamus and indirectly by stimulation of hepatic somatomedin-C synthesis. Somatomedin-C has been shown to stimulate hypothalamic SS release and to inhibit GH secretion. Substance P and glucagon stimulate SS secretion whereas VIP inhibits its secretion.

Prolactin

Regulation of Prolactin Secretion

Human prolactin consists of 199 amino acids and it has three intramolecular disulphide bonds, one more than human growth hormone (GH). It is synthesized, stored and secreted by the lactotrophic cells of the adenohypophysis. The number of lactotroph cells is widely variable (10-30%); it is lowest in men and highest in multiparous women (Asa et al 1982). Normal pituitary contains 100 micrograms of prolactin, only one-fiftieth of the pituitary content of the growth hormone (GH). Thus the pituitary prolactin pool turns over more rapidly than the GH pool. Its secretion is regulated by the hypothalamic prolactin-inhibiting hormone dopamine and by various prolactin releasing factors as shown in Figure 2.8. Prolactin is unique among the anterior pituitary hormones in that it is under tonic hypothalamic inhibition by dopamine produced by tuberoinfundibular dopamine neurons. Dopamine acts by stimulating the lactotroph D2 receptors to inhibit adenylate cyclase and consequently inhibits both prolactin release and prolactin synthesis. Prolactin releasing factors include thyrotropin releasing

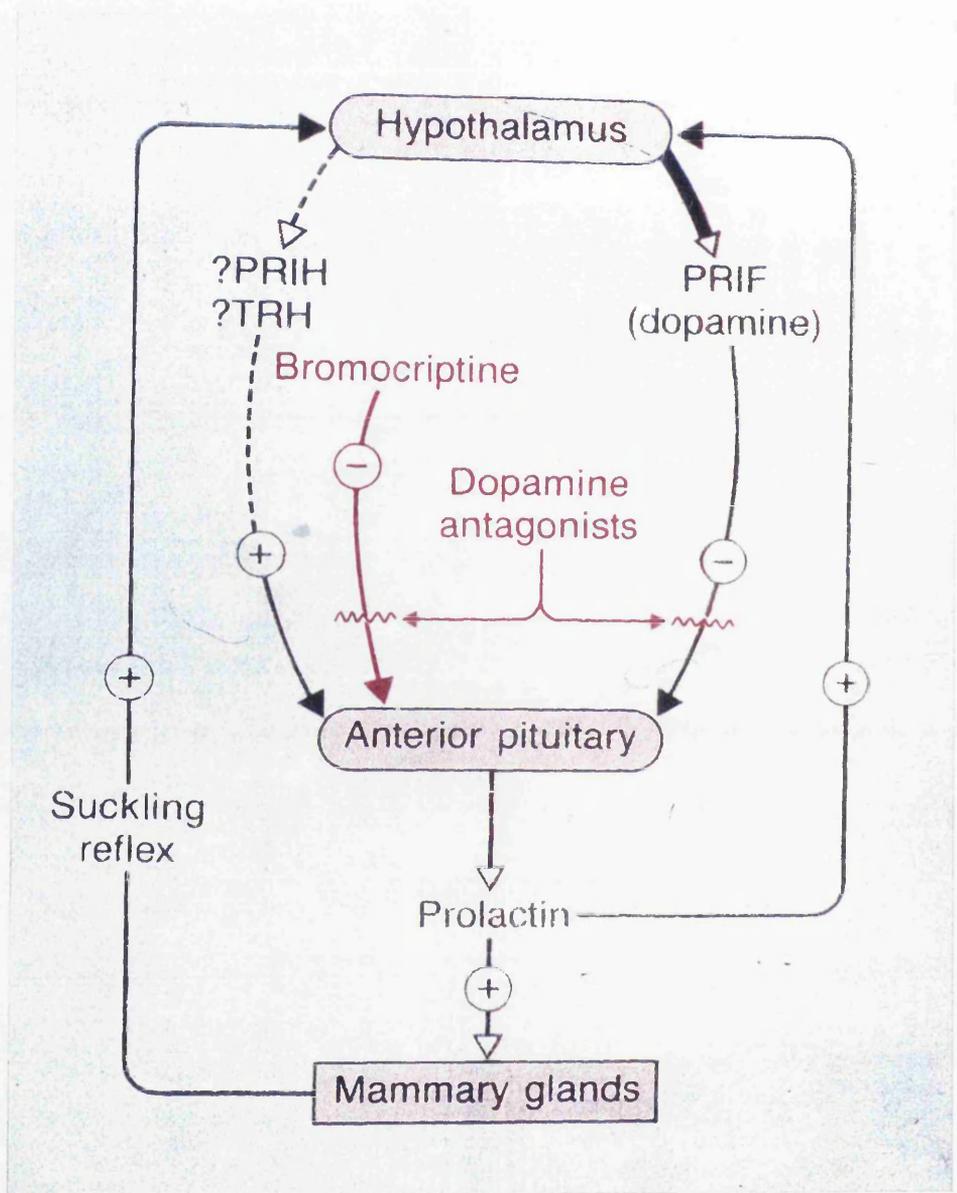


Figure 2.8

Control of prolactin secretion. (TRH=thyrotrophin releasing hormone; PRF=prolactin-releasing factor; PRIF=prolactin release-inhibiting factor.)

hormone (TRH) and vasoactive intestinal peptide (VIP). Prolactin synthesis is also regulated by the effect of estrogen on prolactin gene expression, although that serum prolactin levels are higher in pre-menopausal women than in men.

Regulation of Glucocorticoids (Cortisol) Secretion

Glucocorticoid secretion is regulated by hormone interaction among the hypothalamus, pituitary, adrenal glands (as shown in Figure 2.9) and by neural and other stimuli (Taylor & Fishman 1988; Antoni 1986). Neural stimuli from the brain, as in the response to stress, cause the release into the hypothalamic hypophyseal portal blood of CRH, AVP, and the other agents from the hypothalamic neurons. They are carried to the pituitary, where they stimulate ACTH secretion into the systemic blood. ACTH acts on the adrenal cortex to cause secretion of cortisol and other steroids. The negative feedback loop is completed by the inhibitory effects of glucocorticoids on CRH, AVP, and ACTH synthesis and secretion.

ACTH

ACTH, a 39 amino acid peptide, is synthesized as part of a large, 241 amino acid precursor molecule, proopiomelanocortin (POMC). Human POMC gene is located on chromosome 2. Human pituitary contains approximately 250 micrograms of ACTH, synthesized and stored in corticotroph cells, which are a sub-type of POMC-producing cell line. CRH stimulate ACTH secretion by binding to high affinity CRH receptors on the corticotrophs and by stimulating the accumulation of cyclic AMP (cAMP) which activates protein kinase A. AVP alone is a weak secretagogue of ACTH but acts synergistically with CRH to regulate ACTH release (Debold 1984). The actions of ACTH on the adrenals are mediated through specific high affinity membrane receptors and activation of adenylate cyclase leads to intracellular accumulation of cAMP and thus

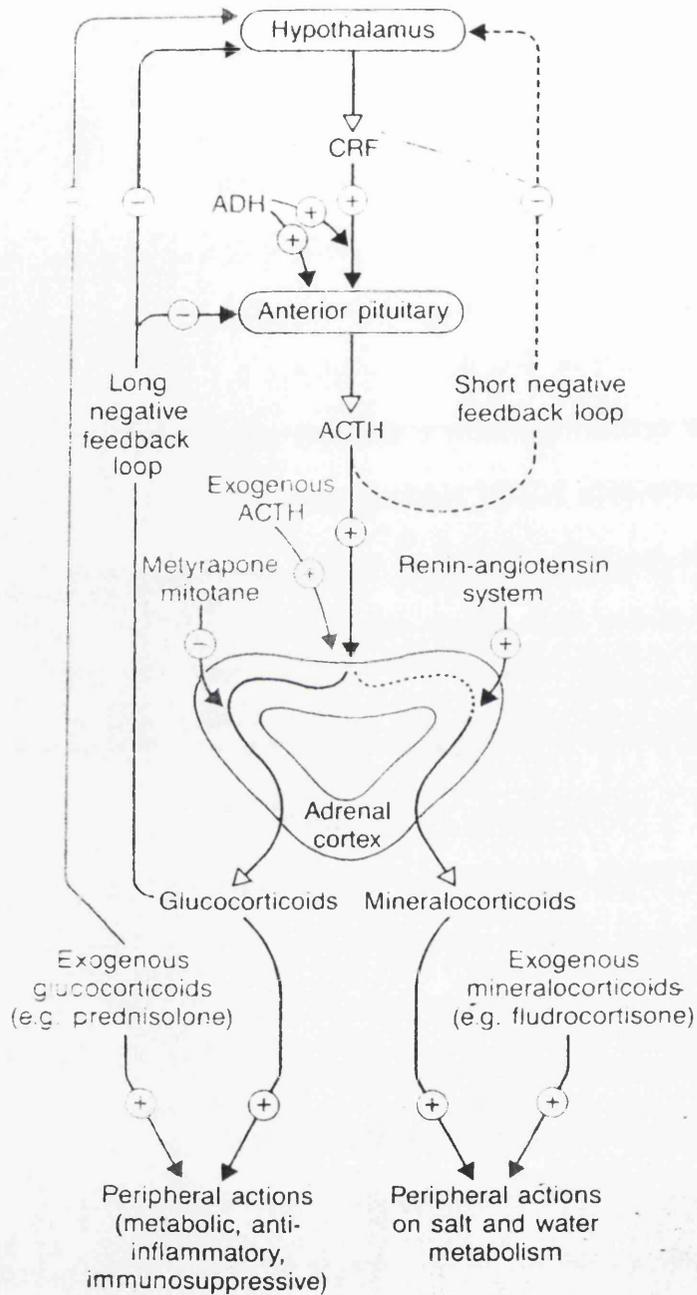


Figure 2.9

Regulation of synthesis and secretion of adrenal corticosteroids. ACTH has only a minimal effect on mineralocorticoid production (indicated by dotted line). (ACTH=adrenocorticotrophic hormone; ADH=antidiuretic hormone (vasopressin); CRF=corticotrophin-releasing factor)

increased protein kinase A activity and phosphorylation of a number of important proteins increase steroidogenesis.

2.3 ASSESSMENT OF NEUROTRANSMITTER (BRAIN RECEPTOR) FUNCTIONS

2.3.1 Neurotransmitters

2.3.1.1 Acetylcholine:

Anatomy & physiology of cholinergic neurones:

In the spinal cord all anterior horn cells supplying nerves to voluntary muscle and all lateral horn cells supplying preganglionic nerves to autonomic ganglia are cholinergic. Ganglion cells giving rise to postganglionic parasympathetic fibres are all cholinergic. Cranial nerves containing voluntary motor fibres and those containing preganglionic parasympathetic fibres also have cholinergic cell bodies. The sympathetic postganglionic fibres to sweat glands are also cholinergic.

The well established cholinergic pathways so far within the brain are neostriatal interneurons, the habenulo-interpeduncular train, septal-hippocampal pathways and cortical neurons.

Central cholinergic neurones have an important role in promoting learning, stimulating thirst, increasing body temperature, promoting aggressiveness and in desynchronizing the EEG in paradoxical sleep and in alert state.

Chemistry of Acetylcholine Synthesis:

Acetylcholine (ACh) is synthesized by the reaction of acetyl-coenzyme A and choline under the influence of the enzyme choline acetyl transferase (CAT).

After synthesis in the cell bodies, it is transported by axoplasmic flow to nerve endings. The substrate for ACh synthesis - i.e. choline, is produced in the liver and is concentrated in cholinergic nerve endings by a high affinity uptake system. Acetyl Co A is a general substrate used in many metabolic reactions, also being generated by cholinergic cells themselves. It is also produced by pyruvate dehydrogenase and glucose. Choline acetyl transferase (CAT) has a molecular weight of about 67,000 and is derived from human neostriatum (Singh & McGeer 1974).

Storage, Release & Turnover and Destruction of Acetylcholine:

After synthesis in the nerve terminals acetylcholine is sequestered from acetylcholinesterase (AChE) by vesicular binding. It is released in a quantal fashion from vesicles and crosses the synaptic cleft. It can be degraded into choline and acetate directly by AChE or reach a receptor site. This sequence of synthesis → storage → release is shown in Figure 2.10.

Cholinergic Receptors:

Two types of cholinergic receptors are well recognised:-

1. Nicotinic.
2. Muscarinic.

Nicotinic receptors include all ganglionic receptors, all receptors of the musculoskeletal system and a few central receptors. Muscarinic receptors occur in smooth muscle

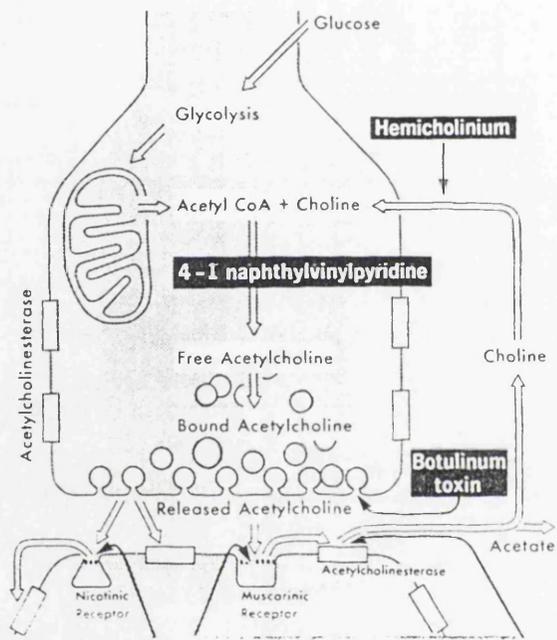


Figure 2.10

Synthesis, storage, release and destruction of acetylcholine.

innervated by postganglionic fibres of the parasympathetic system. This also includes cardiac muscle and certain exocrine glands. Most areas of the brain other than thalamus and cerebellar cortex have muscarinic receptors.

Nicotinic receptors are excitatory and operate by opening ionic channels and hence the action of acetylcholine is rapid through these receptors. Muscarinic receptors operate by the formation of second messenger cyclic guanosine 3', 5' - monophosphate (cGMP) for the action of acetylcholine (Greengard 1976) and hence the action is relatively slow and prolonged.

2.3.1.2 Gamma-Aminobutyric Acid (GABA):

Gamma-Aminobutyric Acid (GABA) is widely known as one of the major inhibitory neurotransmitters in the mammalian central nervous system (Obata and Takeda 1969) and is used by at least 40% of neurons (Guidotti et al 1983).

Anatomical distribution of GABA Pathways:

The brain content of GABA is 200 to 1,000 fold greater than that of other neurotransmitters such as dopamine, noradrenaline, acetylcholine and serotonin. It is widely distributed in the CNS particularly in the cerebellar purkinje cells, golgi and basket cells, hippocampal basket cells, pallidonigral neurons, spinal cord interneurons, cortical and hypothalamic interneurons.

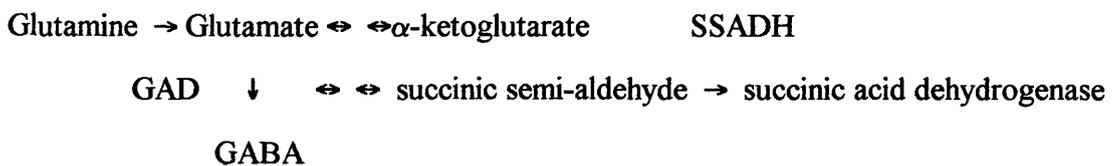
Unlike 5HT or noradrenaline which are projected diffusely from their discrete brain stem and midbrain nuclei, GABA is primarily a transmitter of local inhibitory circuits within the brain. Through these local circuits GABA can rapidly alter the excitability of

primary output neurones and thus regulate neural excitability (Zorumski and Isenberg 1991).

Synthesis, Release and Destruction:

GABA is synthesised from glutamate in nerve endings, probably in vesicles, until it is released, by nerve stimulation. Its destruction is in mitochondria of glial cells, postsynaptic processes as well as nerve endings. The destruction of GABA causes a new molecule of glutamate to form from α -ketoglutarate. The newly synthesised glutamate may be converted to glutamine before it re-enters the nerve ending.

GABA-T



GAD - glutamic acid decarboxylase

GABA-T - GABA α - oxoglutarate transaminase

SSADH succinic semi-aldehyde dehydrogenase

GABA Receptors:

The inhibitory action of GABA is mediated by GABA receptors, which are divided into two types - GABA (A) and GABA (B). It was initially thought that GABA (A) receptor, a bicuculline - sensitive type, forms the GABA - gated chloride ion channel, and that the activation of GABA (A) receptor induces the fast inhibitory postsynaptic potential (IPSP). Subsequently, the presence of GABA (B) receptor which is insensitive to bicuculline and sensitive to baclofen was revealed (Hill & Bowery 1981), and it was found that this receptor is one of the metabotropic types that couple with G-protein.

GABA (B) receptors are located pre and/or post synaptically, and are coupled to various K^+ & Ca^{+2} channels through a pathway involving second messengers. Therefore activation of GABA (B) receptor induces slow inhibitory post synaptic potential (IPSP).

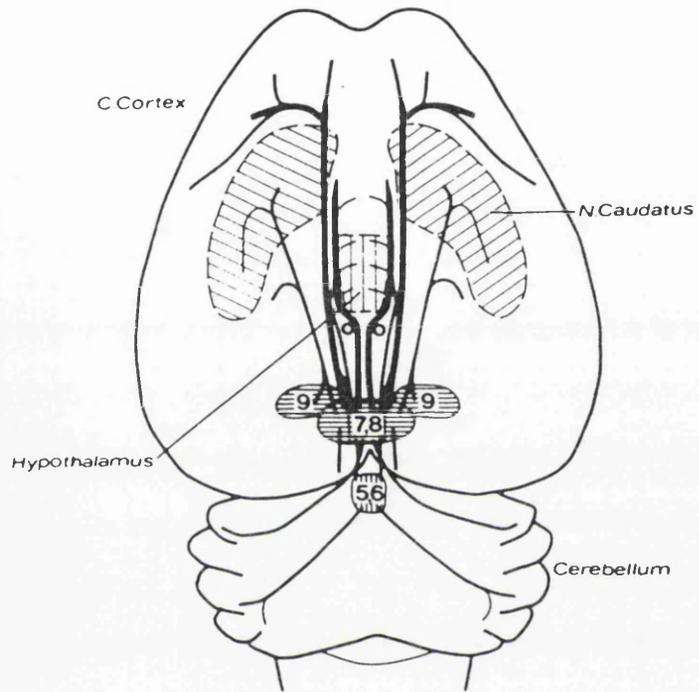
In summary both GABA (A) and GABA (B) receptors have inhibitory roles in the CNS, although these actions are mediated by different molecular mechanisms.

2.3.1.3 Serotonin - [5hydroxytryptamine (5-HT)]:

Anatomy of serotonergic neurons.

Cell bodies of 5HT neurons are located in the raphe and reticular systems of the brain stem. The neurons of the caudal raphe nuclei project into this spinal cord, and those of the rostral raphe nuclei give rise to ascending pathways to the diencephalon and telencephalon as shown in Figure 2.11a & 2.11b (Fuxe & Johnsson 1974). There are important functional as well as anatomical differences among different rostral raphe nuclei and their projection systems which provide dual innervation of different brain regions, sometimes with opposing functional effects (Blier et al 1990, Murphy 1991). The divergence of terminals suggests that the role of serotonin neurons is to exert a generalised tonic effect rather than to have a highly selective action on a limited group of neurons.

ANATOMY OF SEROTONIN NEURONS



Horizontal projection of ascending serotonin pathways. (From Fuxe and Johansson 1974.)

Figure 2.11a

Horizontal projection of ascending serotonin pathways, (Adopted from Fuxe and Johansson 1974)

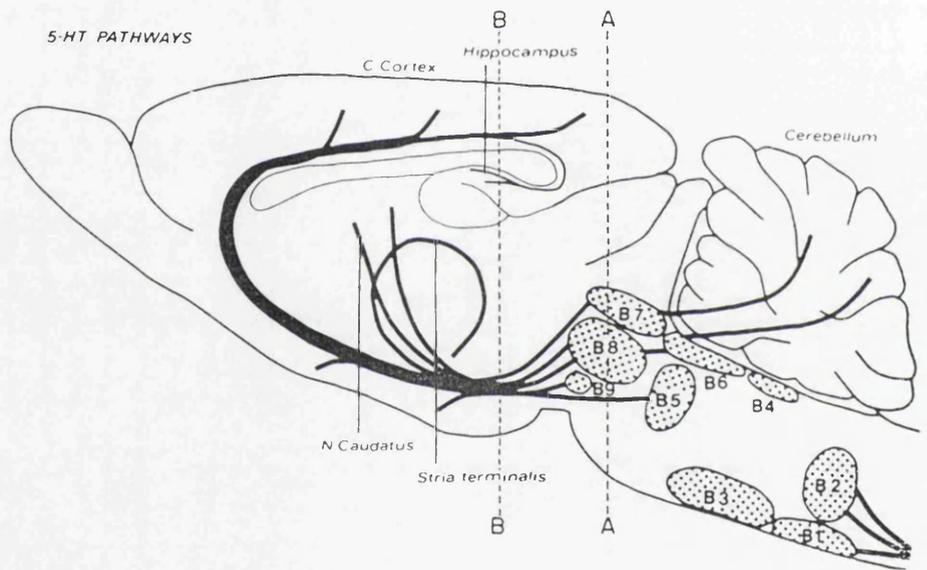
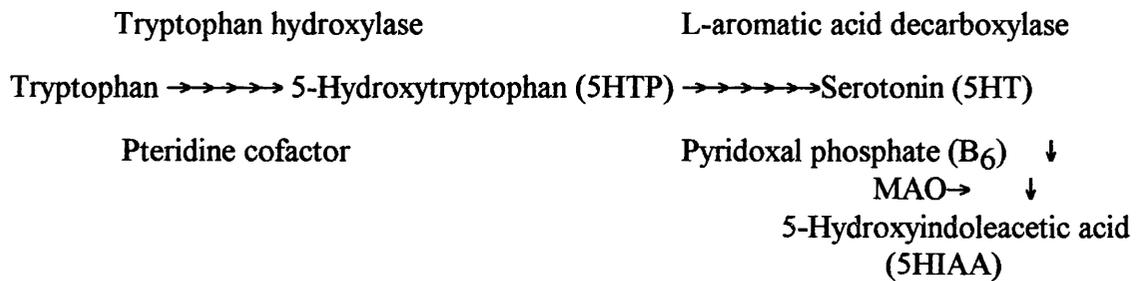


Figure 2.11b

Schematic diagram of central serotonergic cell groups and projections in sagittal sections from rat brain. (Adopted from Fuxe and Johansson 1974)

Metabolism:

The sequence of reactions by which serotonin is created and destroyed is as following. The dietary amino acid tryptophan is converted to 5-hydroxytryptophan (5HTP) by the enzyme tryptophan hydroxylase. 5HTP decarboxylase then converts this intermediate amino acid to serotonin. Serotonin is metabolised both pre and post synaptically by the enzyme monoamine oxydase (MAO) which produces the inactive metabolite 5-hydroxyindolacetic acid (5HIAA).



5-HT Receptors:

Serotonin (5HT) receptors exist in many different tissues (brain, gut, blood vessels and many others) and on many different cell types (e.g. neurons, platelets, smooth muscle cells). Two types of brain serotonin receptors, 5HT₁ and 5HT₂ were defined in 1979 (Peroutka & Snyder). Subsequently, additional subtypes were recognised and classified according to their second messenger associations. (Humphrey et al 1993). This approach has led to the division of 5HT receptors into those linked to adenylate cyclase (5HT_{1A}, 5HT_{1B}, 5HT_{1D}, 5HT₄), those linked to phosphatidyl inositol system (5HT_{2A}, 5HT_{2B}, 5HT_{2C}), and those linked directly to ion channels (5HT₃). 5HT play a role in thermoregulation. 5HT_{1A} agonist produces hypothermia whereas 5HT_{1B} and 5HT₂ agonists appear to produce hyperthermia (Martin et al 1988, Gudelsky et al 1988). Others have also reported involvement of 5HT_{1B} receptors in sexual behaviour (Olivier et al 1987, Mendleson 1988). Cardiovascular effects of 5HT depends upon the species

of animal, the vascular bed under study and the dose of drug (Saxena et al 1987). 5HT₂ mechanisms may be involved in peripheral vasoconstriction whereas coronary and cerebral vasoconstriction is 5HT₁ mediated (Van Houtte 1987). Hypotensive effect of serotonin is mediated via inhibition of central vasomotor loci, which involves 5HT₁ receptors and inhibition of release of norepinephrine from postganglionic sympathetic neurons which also involve 5HT₁ like receptors (Van Zwieten 1987). 5HT can also release vasodilator substances from vascular endothelium (Van Zweiten 1987) and also regulates sleep-waking cycle (Koella 1988), and plays a part in the aetiology of anxiety disorders (Iverson 1984, Eriksson 1987), depression (Coppen & Doogan 1988) and migraine (Fozard & Gray 1989).

2.3.1.4 Dopamine:

Anatomy of dopamine neuron:

Dopaminergic neuronal system arises from a series of cell bodies in the brain stem and gives rise to three dopaminergic pathways, i.e. nigrostriatal (Dahlstrom and Fuxe 1964 a,b), mesolimbic - mesocortical as shown in Figure 2.12a & 2.12b (Ungerstedt 1971) and tuberoinfundibular (Dahlstrom and Fuxe 1964 a,b). These pathways are shown in horizontal and sagittal projections in figure 2.12a & 2.12b. In addition, there are dopaminergic interneurons in the brain stem superior cervical ganglion (Bjorklund et al 1970), hypothalamus (Lindvall & Bjorklund 1974), retina (Ehinger 1977), olfactory bulb (Halasz et al 1977) and carotid body (Bolme et al 1977).

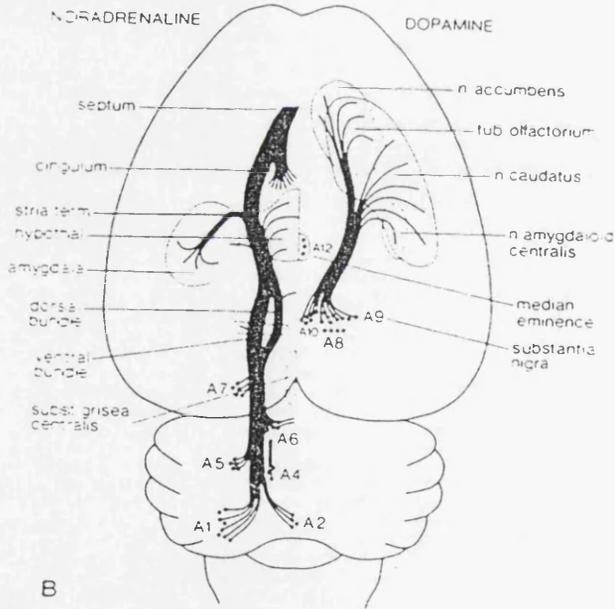
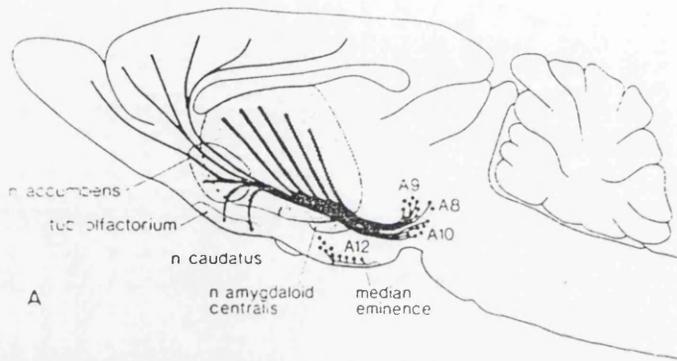
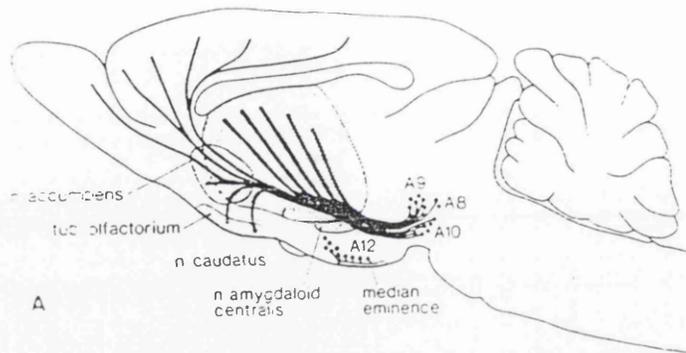
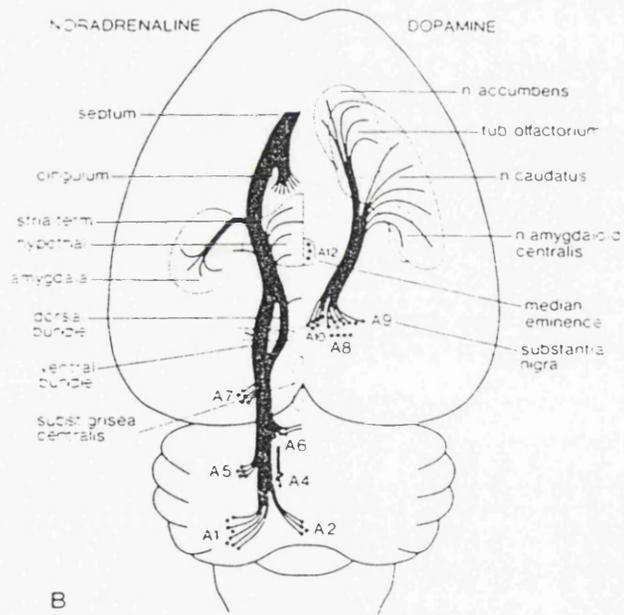


Figure 2.12a

Sagittal projection of dopamine pathways in rats. Striped areas indicate dense terminal fields. (Adopted from Ungerstedt 1971).



A



B

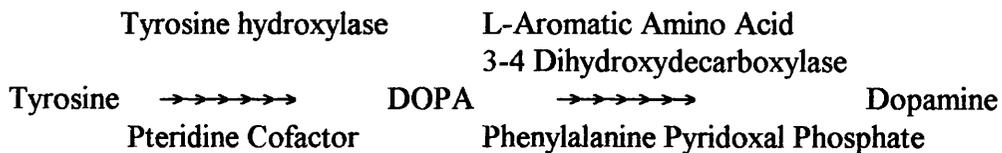
Figure 2.12b

Horizontal projections of the ascending dopamine and noradrenaline pathways.

(Adopted from Ungerstedt 1971).

Dopamine synthesis, storage, release and destruction:

The synthesis of dopamine takes place in the cytoplasm of the nerve endings from tyrosine under the action of two enzymes, tyrosine hydroxylase and 3,4-dihydroxydecarboxylase. The synthesised dopamine is either stored in vesicles or destroyed presynaptically by intrasynaptosomal monoamine oxidase (MAO) or released into the synaptic cleft. After release it can be recaptured by the nerve ending or destroyed by MAO or catechol-o-methyl transferase (COMT).



Physiological effects of dopamine:

Dopamine is associated with initiation and execution of movements, plays an important role in thought organisation and has been implicated in a number of behaviours including stereotype, eating and drinking. It also has an important governing influence on the endocrine system, particularly in promoting the release of GH and inhibiting the release of PRL.

Dopamine receptors:

At present three types of dopamine receptors have been identified in the brain, namely D1, D2 and D3. The D1 type stimulates adenylate cyclase whereas the D2 type inhibits this enzyme (Stoof & Keibian 1981). The D3 receptor has recently been cloned with

higher concentrations in limbic regions and ventral striation (Sokoloff et al 1990). D2 receptors are located on the striatal cells and terminals of the corticostriate projections but also on the synaptic terminals, soma and dendrites of the dopaminergic neurons of the substantia nigra pars compacta (Aghajanian & Bunney 1977).

2.3.1.5 Noradrenaline:

Anatomy of noradrenaline neuron:

Ungerstedt (1971) with histofluorescence methods described two main ascending noradrenergic pathways, a dorsal and a ventral bundle as shown in Figure 2.12b & 2.13. Ventral noradrenergic pathway gathers fibres from the cell bodies in the medulla oblongata and pons. The axons ascend in the midreticular formation and turn ventromedially along the medial lemniscus and continue rostrally mainly within the medial forebrain bundle. In its route it gives rise to noradrenergic nerve terminals in the lower brain stem, mesencephalon, and diencephalon. The dorsal ascending noradrenergic pathway has its cell bodies in the locus coeruleus. Their axons ascend in the dorsal tegmentum and at the diencephalic level turn ventrally to join the ascending dopamine axons in the medial forebrain bundle. Terminals are found in diencephalon, limbic system, and cortex. The locus coeruleus also gives rise to noradrenergic terminals in the cerebellum and lower brain stem nuclei. There are also descending pathways to ventral and dorsal horn, and sympathetic lateral columns.

In the peripheral nervous system noradrenergic fibres are the postganglionic gray rami arising from the paravertebral ganglia which contain their cell bodies. They receive their preganglionic fibres from the 12 thoracic and upper 2 lumbar segments.

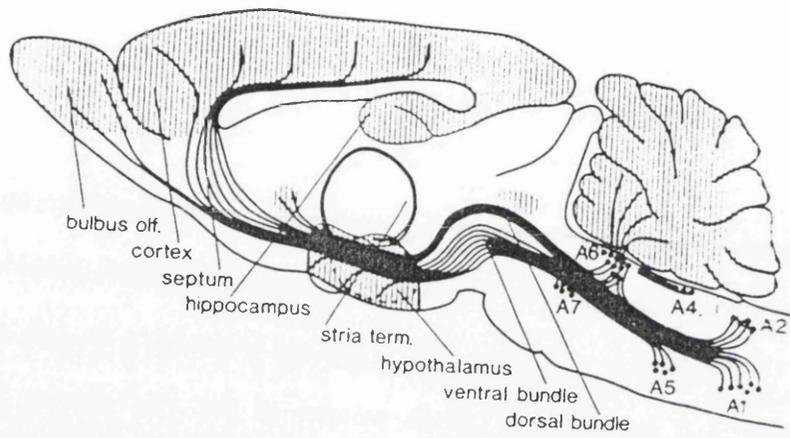
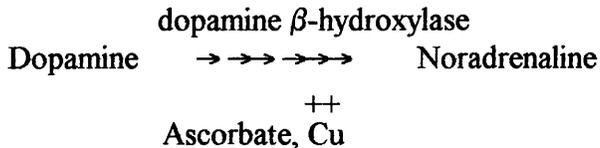


Figure 2.13

Noradrenaline projections by histochemistry; sagittal projection of the ascending NA pathways traced by fluorescent histochemical method. Striped areas indicate major nerve terminal fields. (Adopted from Ungerstedt 1971)

Synthesis, storage, release and inactivation:

Noradrenergic neurons contain dopamine β -hydroxylase which hydroxylates the ethylamine side chain of dopamine and converts it into noradrenaline.



Noradrenaline is metabolised by MAO and COMT. The peripheral products of its metabolism are normetanephrine, 3-4-dihydroxymandelic acid, and vanillylmandelic acid (MHMA or VMA) and of central metabolism are 3-4-dihydroxyphenylglycol and 3-methoxy 4-hydroxyphenylglycol (MHPG). After synthesis it is stored in storage vesicles, released by nerve stimulation into synaptic cleft and inactivated by reuptake into the nerve endings.

Adrenoceptors:

Two types of adrenoceptors are well recognised:-

1. Alpha (α)
2. Beta (β).

Alpha receptors are further subclassified into α_1 and α_2 , likewise Beta receptors are subdivided into β_1 and β_2 .

α_1 adrenoceptors are located post-junctionally and α_2 -receptors which are mainly located pre-junctionally on noradrenergic nerve terminals functions as autoreceptors by regulating the release of noradrenaline (NE) through a negative feedback mechanism (Nyback et al 1975; Langer et al 1980). Whereas α_2 postsynaptic receptors on

activation may result in the stimulation of growth hormone release (Terry and Martin 1981).

All adrenoceptors are members of the receptor superfamily which are coupled to G proteins. Each adrenoceptor consists of a single polypeptide chain of 400-560 amino acids which crosses the cell membrane 7 times. The amino terminal (NH₂) is extracellular and the carboxyl terminal intracellular. Each transmembrane segment forms a hydrophobic- α -helix. All receptors interact with G. proteins at an intracellular site. Different receptors interact with different G. proteins, for example β_1/β_2 are coupled to Gs, α_2 coupled with Gi/Go and α_1 to Gq proteins. Both Gs and Gi act to modulate the activity of adenylate cyclase. Gs stimulate whereas Gi inhibit the activity of this enzyme. Activated Gq activates Inositol 1,4,5-triphosphate system. In a number of tissues Gi proteins can also modulate the activity of certain ion channels without involvement of intracellular second messengers. α_2 -adrenoceptors also contraction of vascular smooth muscle by increasing calcium influx through L-type calcium channels without activating second messengers.

2.3.2 Neuroendocrine Challenge Tests

With the development of the radioimmunoassay during the late 1950s and the 1960s it became possible to measure endocrine function in quantitative terms in an accurate way. The basis of a radioimmunoassay is competitive inhibition of the binding of labelled hormone to antibody by unlabelled hormone contained in standards or in unknown samples (Yalow 1985). The broad normal range for some plasma hormone concentrations makes the interpretation of one value in an individual unreliable, if the previous normal value for that person is unknown. In addition, there are subtle degrees of endocrine organ dysfunction that can be compensated for under basal conditions.

Thus the cortisol levels in plasma and the cortisol secretion rates can be normal in patients with partial adrenocortical insufficiency as the result of increased secretion of corticotrophin (ACTH). Dynamic endocrine tests provide additional information to that obtained from measurements of a single hormone and can uncover these less obvious abnormalities in endocrine function. Such tests are based on either the stimulation or suppression of endogenous hormone production.

Stimulation tests are used when hypofunction of an endocrine organ is suspected and are designed to perturb the endogenous control mechanisms so as to assess the reserve capacity to form and secrete hormone. This assessment is done in either of two general ways. A trophic hormone can be administered to test the capacity of the target organ to increase hormone production. The trophic hormone can be a hypothalamic-releasing factor, for example TRH, and the capacity of the target organ is assessed by measuring TSH. Alternatively, a stimulatory test may be performed by causing an increase in the secretion of an endogenous trophic hormone or stimulatory factor in measuring the effect of the procedure on a target hormone.

For the studies in this thesis we utilised stimulatory tests that were designed to increase the endogenous trophic factors in the hypothalamus through central neurotransmitter activation and to measure specific aspects of pituitary reserve. These hormonal responses to drug challenge provide an important bioassay for the evaluation of neurotransmitter function for evaluating the integrity of its pathway and the sensitivity of its receptor systems. Included in this group are the growth hormone responses to pyridostigmine, bromocriptine, dexamethasone, desipramine and baclofen; and prolactin responses to buspirone. This neuroendocrine approach to assess neurotransmitter function is heavily dependent on the development of selective drugs for challenging specific receptor systems.

Pharmacological Criteria For Neuroendocrine Tests Of Neurotransmitter Function:

When the following criteria are satisfied it is reasonable to assume that the hormone response to a particular drug results from the stimulation of a specified receptor: Firstly, a similar hormonal response should result from administration of other drugs that stimulate the specified neurotransmitter system. Secondly, it should be possible on each occasion to block this response with drugs which selectively block receptors of this system. Thirdly, antagonists at other neurotransmitter systems should not inhibit the hormonal response. Fourthly, the response must be produced either by drugs that only cross the blood brain barrier or if mediated at peripheral sites can be shown by animal studies to be at the median eminence (Checkley, 1980). Pharmacological probes can stimulate either receptor sites or alter the release of reuptake of neurotransmitter. In this thesis an attempt is made to analyse the regulation of different neurotransmitter systems and their influence on the functional integrity of the HPA axis in patients with CFS.

2.4 OUTLINE AND AIMS OF STUDIES

Due to overlap between the symptoms of CFS and depression, the separation of these two entities is sometimes difficult if not impossible. Abnormalities in the hypothalamic-pituitary-adrenal axis (HPA), in addition to disturbed neurotransmitters function, have been the most consistently demonstrated biological markers in depressive illness (Dinan 1995); whereas very few studies have been conducted into the biology of CFS.

The objective of these studies was to examine neuroendocrine aspects of monoaminergic (serotonergic, adrenergic, dopaminergic), GABAergic and cholinergic function in an attempt to explore the underlying neurobiological mechanisms in this illness.

The first study was designed to test the hypothesis that serotonergic neurotransmission is enhanced in CFS (Bakheit et al 1992). Hormonal responses to 5-HT_{1A} agonist buspirone were examined in CFS, compared to a healthy control group. In the second study HPA function was examined by looking at ACTH and cortisol responses to 5-HT_{1A} receptor agonist ipsapirone.

In a subsequent study to assess the function of cerebral steroid receptors, dexamethasone-induced growth hormone release was measured in two phases, before and after the administration of metyrapone. Responses to this battery of tests were compared to responses in healthy and depressed individuals.

Next, to assess the functioning of acetylcholine and adrenaline neurotransmission we measured hormonal (GH) responses to the anticholinesterase pyridostigmine and the monoamine reuptake inhibitor-desipramine respectively in patients with CFS and healthy subjects.

Finally, dopaminergic and GABAergic function was examined by measuring growth hormone responses to the dopamine agonist bromocriptine and the GABA (B) agonist baclofen respectively, in patients with CFS compared with healthy controls.

GENERAL SUBJECTS AND METHODS:

3.1 SUBJECTS

Group 1: Patients with Chronic Fatigue Syndrome.

All patients with chronic fatigue syndrome were seen at the Institute of Neurological Sciences CFS out-patient clinic, Glasgow, and were hospitalised as in-patients. All patients were selected according to the criteria outlined in the introduction of this thesis and they also fulfilled the CDC criteria laid down by Fukuda et al in 1994. Those with co-existing depression were excluded. Essentially, patients meeting the CDC criteria had to have persisting or relapsing, debilitating fatigue for at least six months in the absence of any medical diagnosis which would explain the syndrome. Laboratory tests which include ESR, complete blood count and differential, protein electrophoresis, serum electrolytes, glucose, creatinine, blood urea, serum calcium and phosphate, liver function tests, muscle enzymes, urinalysis, chest x-rays, thyroid function tests, antinuclear antibodies screen were carried out to exclude any other medical cause for their fatigue.

Group 2: Patients with major depressive illness.

All patients with major depression were seen at St. Bartholomew's Hospital, psychiatric out-patient clinic and were admitted to the hospital for neuroendocrine studies. All patients were seen by psychiatrists and fulfilled the criteria for major depression as defined by the Diagnostic and Statistical Manual of Mental Disorders, Third Edition, Revised (DSM-III-R). Depression was rated using the Hamilton Rating Scale for Depression (Hamilton 1960) and those with a score greater than 17 were included. These patients like those in Group 1, were extensively studied as were in-patients.

Group 3: Healthy controls.

These were age and sex matched staff and student volunteers from both hospitals. All subjects had a full physical examination and psychiatric interview. None had a past or current history of chronic fatigue, psychiatric illness, neurological, endocrine, cardiovascular, renal, hepatic or cerebrovascular disease. None had a history of any alcohol consumption or illicit drug use.

All patients and control subjects were medication free for at least three months. Females were tested in the early follicular phase of menstrual cycle. All patients gave written, informed consent, and the studies were approved by the Ethics Committees in the Southern General Hospital, Glasgow, and St. Bartholomew's Hospital, London.

3.2 PROCEDURE

Subjects were given all tests at the same time of the day, i.e. 9.00 am. The tests were performed in the morning after an overnight fast. They had a cannula inserted in a forearm vein at 8.30 am and they were allowed to relax for 30 minutes. The cannula was kept patent by flushing with heparin. Serial samples were taken for estimation of either growth hormone, prolactin, cortisol or ACTH at 0, +30, +60, +90, +120 and +180 minutes after the administration of different pharmacological agents in different studies. Details are given in the relevant sections. The samples were immediately centrifuged and stored at -80° C until analysis. Blood pressure and heart rate were measured at 15 minute intervals throughout.

3.3 HORMONAL ASSAYS

Growth hormone was measured in the Department of Biochemistry, Royal Infirmary, Glasgow using an "in house" immunoradiometric assay employing UK9 as primary working HGH standard. The sensitivity of the method is 0.2 mU/l (CV <22%) with a between batch variation of 5% over the working range 5 to 40 mU/l.

Prolactin concentrations were also measured by immunoradiometric assay. The assay was standardised against the National Institute for Biological Standards and Control 3rd International Standard 84/500, and the within and between batch coefficients of variation were 3% and 6% respectively over the concentration range 200-3000 mU/l.

ACTH was measured using a commercially available 2-site immunoradiometric assay. Intra and interassay coefficients of variation were 6% and 10% respectively. The reliable lower limit of detection was 4.4 Pmol/l (20 ng/l). Cortisol was measured by double antibody RIA. The sensitivity of the method is 30 nmol/l with a between batch variation of <10% over the range 100 to 1200 nmol/l.

3.4 STATISTICAL ANALYSIS

Unless stated otherwise the results are expressed as mean \pm SEM and statistically analysed by means of statgraphics (Statistical Graphics Corporation 1987). Endocrine responses over time to drug were evaluated by analysis of variance (ANOVA) with repeated measures and Tukey's post hoc comparisons were applied. This analysis was performed to assess statistical significance of the main effects of group (patients vs controls), and time (changes over several time points). To evaluate whether the patients with chronic fatigue syndrome and controls reacted differently to drug, the interaction of group x time was examined.

The comparisons of basal and drug induced hormone release [Delta Δ = difference between baseline and maximum subsequent increase after drug administration] were done by student's t-test and one-way analysis of variance.

3.5 DRUGS ADMINISTERED

Ipsapirone

Ipsapirone, a centrally acting pyrimidinyl piperazine derivative, is a selective 5HT_{1A} agonist with only negligible affinity for 1B, 1C, 1D, 2 and 3 subtypes and only moderate affinities to α -adrenergic and dopamine D₂ sites.

It has been shown to produce a dose dependent increase in ACTH and cortisol which is blocked by 5HT_{1A} antagonists (Lesch et al 1990 and Koenig et al 1988). Ipsapirone induced ACTH and cortisol release appears to be mediated through 5HT_{1A} receptor sites on the CRH-synthesizing neurons in the paraventricular nucleus of the hypothalamus because selective surgical or neurotoxic lesions of ascending serotonergic fibres or the raphe nuclei do not prevent the ACTH and corticosterone response to 5HT releasing compounds or 5HT agonists in rats (Van de Kar et al 1985).

Baclofen

Baclofen is a selective GABA (B) receptor agonist which freely crosses the blood brain barrier (Naik et al 1976). It has both pre and post synaptic effects. Pre synaptic effects comprise the reduction of the release of both excitatory and inhibitory transmitters, whereas post synaptic GABA (B) receptor mediated inhibition occurs by activating potassium channels through a second messenger pathway involving arachidonic acid.

Hypothalamic GABA (B) receptors are involved in the modulation of human growth hormone secretion (Koulu et al 1979, Passariello et al 1982, Monteleone et al 1988). Therefore measurement of GH responses to acute administration of baclofen is an important tool to assess GABAergic function.

Buspirone

Buspirone is a piperazinyll compound which has diverse behavioural effects in animals and humans and is now being used in the management of anxiety disorders. It is a 5HT_{1A} agonist as well as dopamine antagonist, releases prolactin in a dose dependent manner (Meltzer et al 1983). It can also cause dizziness, nervousness, headaches and nausea.

Dexamethasone

Dexamethasone is a synthetic glucocorticoid with a higher affinity (7 fold) than cortisol for the intracellular type 2 glucocorticoid receptors. In contrast, the type 1 receptor mediated activities are negligible compared with their glucocorticoid activities. It is absorbed within 30 minutes after oral administration and its half life is 4 hours with considerable interindividual variation. It is widely used for both diagnostic and therapeutic purposes.

Despiramine

Despiramine is a tricyclic antidepressant, is a monoamine - reuptake inhibitor and thus indirectly stimulate the alpha 2 adrenoceptor and hence produce an increase in GH

levels. This effect of despiramine is blocked by the alpha 1 and alpha 2 antagonist phentolamine but not by alpha 1 antagonist prazosin (Laakmann et al 1986).

Pyridostigmine

Pyridostigmine is an anticholinesterase and is mainly used in the treatment of myasthenia gravis to increase neuromuscular transmission. It inhibits the cholinesterase enzyme which breaks down acetylcholine in the synaptic cleft, thus increasing both the amount and duration of action of the transmitter at the receptor sites. It can cause nausea, salivation, diarrhoea and colic. Pyridostigmine brings about an increase in growth hormone secretion from somatotrophs by inhibiting somatostatin release (Ross et al 1987).

Bromocriptine

Bromocriptine is an ergot derivative, stimulates D2 receptors and antagonises D1 receptors. It is used in the treatment of Parkinson's disease, acromegaly and hyperprolactinemia. The common side effects of this drug are nausea, postural hypotension, dizziness, headache and vomiting.

Patients with CFS examined in each study were not the same individuals. In each study different patients were recruited.

3.6 STUDY 1: 5-HT mediated prolactin release

Methods and subjects

Subjects

60 subjects participated in this study; 30 healthy subjects and 30 patients with CFS. All patients fulfilled the strict criteria for the CDC revised case definition. The CFS group consisted of 15 males, mean age 42 years, and 15 females, mean age 44 years.

15 men, mean age 40, and 15 women, mean age 46 years, were included in this control comparison group. None had a personal or family history of psychiatric disorder. All females were tested in the follicular phase of their menstrual cycle. All subjects gave fully informed consent.

Procedure

The test was carried out at 9.00 am following an overnight fast. A cannula was inserted into the antecubital vein at 8.30 am and blood samples for baseline prolactin estimation taken 30 minutes later. A standard dose of buspirone 60 mgs was given orally at 9.00 am to all subjects. Further blood samples were taken hourly for four hours. Blood samples were centrifuged immediately and stored at - 80° C until analysis.

Hormonal assay

Prolactin concentrations were also measured by immunoradiometric assay. Assay were standardised against the National Institute for Biological Standards and Control 3d

International Standard 84/500, and the within and between batch coefficients of variation were 3% and 6% respectively over the concentration range 200-3000 mU/l.

Statistical analysis

Results were expressed as mean \pm SEM and statistically analysed as follows. To calculate differences in baseline prolactin and Δ prolactin (difference between baseline and maximum value after buspirone administration) levels between patients with chronic fatigue syndrome and healthy volunteers, student's t-test was employed.

Two-way ANOVA with repeated measure and post hoc Tukey comparisons were applied to compare prolactin response curves to buspirone in healthy subjects and patients with chronic fatigue syndrome.

3.7 STUDY 2: Serotonin mediated activation of the hypothalamic-pituitary-adrenal axis in CFS:

Subjects

14 patients - 6 males, mean age 34 years, and 8 females, mean age 36 years, fulfilling the revised CDC criteria for CFS together with 14 age and sex matched healthy comparison subjects were recruited. The latter had a full physical examination and psychiatric interview. None had a personal or family history of psychiatric illness. Pre-menopausal females were tested in the follicular phase of the cycle. All subjects were medication free for at least 6 weeks before the study. None had a history of alcohol consumption or illicit drug use.

Procedure

The test was carried out at 9.00 am following an overnight fast. They had a cannula inserted in an antecubital vein at 8.30 am and they were then allowed to relax for 30 minutes. Baseline blood for ACTH and cortisol estimation was drawn. The cannula was kept patent by flushing with heparinase. A standard dose of ipsapirone 20 mgs was administered orally at 9.00 am. Further blood for ACTH and cortisol estimation was drawn at +30, +60, +90, +120 & +180 minutes. The samples were immediately centrifuged and stored at -80° C until analysis. Blood pressure and heart rate were measured at 15 minute intervals throughout.

Hormonal assay

ACTH was measured using a commercially available two site immunoradiometric assay. This is a non-extraction assay supplied by the Nichols Institute, San Juan Capistrano, CA. Intra and interassay coefficients of variation were 6% and 10% respectively. The reliable lower limit of detection was 4.4 pmol/L (20 ng/L). Cortisol was measured by double antibody RIA. The sensitivity of the method is 30 nmol/L with a between batch variation of less than 10% over the range 100 to 1200 nmol/L.

Statistical analysis

ACTH and cortisol responses were measured as the maximum level post ipsapirone relative to baseline (Δ ACTH and Δ cortisol). Two-tailed student t-test were used to compare means. A two-way repeated measures ANOVA with planned comparisons was used to compare the hormone responses over time.

3.8 STUDY 3: Dexamethasone - induced growth hormone release: Evidence for cerebral steroid receptor resistance.

Subjects

36 subjects participated in this study: 12 healthy control, 12 with major depression and 12 with CFS. Group 1 - Patients with CFS fulfilled the strict criteria for the Centres of Disease Control (CDC) revised case definition. All known causes of fatigue were eliminated, and patients had an extensive clinical work-up at in-patients. This group consisted of 6 men, mean age 38 years, and 6 women, mean age 40 years. Group 2 - Patients with major depressive illness fulfilled the DSM - 111-R criteria for their diagnosis and all had a Hamilton Depression Rating Score greater than 17. This group also consisted of 6 males, mean age 43 years, and 6 females, mean age 40 years. Group 3 - Healthy comparison subjects: This group also included 6 males, mean age 35 years, and 6 females, mean age 33 years. All subjects had a full physical examination together with routine haematology, biochemistry and thyroid function screening. No subject was admitted to the study who was taking any medication. Females were tested in the early follicular stage of menstrual cycle. All patients gave written informed consent.

Procedure

The test was carried out in two phases and commenced in the morning at 9:00 am following an overnight fast. They relaxed for 15 minutes following the insertion of a cannula and heparin bung in a forearm vein. In the first phase dexamethasone 4 mgs was given orally at 0 minute and blood samples were drawn at 0, +60, +120, +180 & +240 minutes for growth hormone estimation. In the second phase of the study after a

week interval the same subjects were given metyrapone - 500 mgs, 6 hourly for 24 hours and then the dexamethasone test repeated as in the first phase.

Hormonal assay

Growth hormone was measured in the Department of Biochemistry, Royal Infirmary, Glasgow using an "in house" immunoradiometric assay employing UK9 as primary working HGH standard. The sensitivity of the method is 0.2 mU/l (CV <22%) with a between batch variation of 5% over the working range 5 to 40 mU/l. Cortisol was measured by double antibody RIA. The sensitivity of the method is 30 nmol/l with a between batch variation of <10% over the range 100 to 1200 nmol/l.

Statistical analysis

One-way analysis of variance was used to compare baseline growth hormone, Δ GH₁ (difference between baseline values and maximum increase post DEX in phase 1), Δ GH₂ (difference between baseline and maximum increase post DEX in phase 2 after metyrapone) values in patients with chronic fatigue syndrome, depression and healthy volunteers. Two-way ANOVA with repeated measures and post hoc Tukey comparison were applied to compare GH response curves to DEX during phase 1 and 2 in healthy subjects and patients with chronic fatigue syndrome and depression.

3.9 STUDY 4: Desipramine induced GH release

Subjects

Fourteen patients and fourteen healthy controls gave fully informed consent to participate in this study. CFS group consisted of six male, mean age 46 years, and eight females, mean age 35 years, who fulfilled the revised CDC criteria for chronic fatigue syndrome. All known causes of chronic fatigue were eliminated, and the the patients had an extensive clinical work up as in-patients. This included routine haematology, biochemistry and thryoid function screening.

Our control group, six men and eight women (mean age 43 and 38 years respectively) were physically healthy and had no family or personal history of psychiatric disorder.

Premenopausal female subjects were tested in the follicular phase of menstrual cycle. None of the patients or controls was on any medication for at least six weeks before the study.

Procedure

Test was carried out at 9.00 am following an overnight fast. They relaxed for 15 minutes following the insertion of a cannula and herparin bung in a forearm vein. Baseline sample was taken at 0 minute and thereafter at +60, +90, +120 and +180 minutes for estimation of growth hormone. A standard dose of desipramine 75 mgs was administered orally at 0 minute. All subjects remained in a supine position throughout the procedure. The samples were immediately centrifuged and stored at -80° C until analysis.

Hormone analysis

Growth hormone was measured in the Department of Biochemistry, Royal Infirmary, Glasgow using an "in house" immunoradiometric assay employing UK9 as primary working HGH standard. The sensitivity of the method is 0.2 mU/l (CV <22%) with a between batch variation of 5% over the working range 5 to 40 mU/l.

Statistical data

Growth hormone responses over time to desipramine challenge were evaluated by analysis of variance (ANOVA) with repeated measures and Tukey's post hoc comparisons were applied. Δ growth hormone (difference between baseline values and the maximum increase post desipramine administration) measures were also used to compare responses in both groups. Two-tailed student's t-tests were used. Results were expressed as mean \pm SEM.

3.10 STUDY 5: Pyridostigmine induced growth hormone release:

Subjects

Twenty eight subjects participated in this study: sixteen healthy subjects and twelve patients with CFS. All patients fulfilled the strict criteria for the Centres of Disease Control (CDC) revised case definition. The CFS group consisted of six men mean age 31 years and six women mean age 36 years.

Eight men, mean age 32 years and eight females mean age 33 years, were included in the control subjects. None had a personal or family history of psychiatric disorder.

All female subjects were tested within the first ten days of menstrual cycle. All patients and healthy control subjects gave fully informed consent.

Procedure

All patients were hospitalised the night before the test. The test was commenced in the morning following an overnight fast. An intravenous cannula was inserted into a forearm vein at 8.30 am and they were allowed to relax for 30 minutes. Baseline blood for growth hormone estimation was drawn at 9.00 am. The cannula was kept patent by flushing it with heparinse (0.5 ml, 50 units) after samples of blood were taken and the first 2 mls extracted at each time point was discarded. Pyridostigmine 120 mgs orally was administered at 0 minute, i.e. at 9.00 am and further blood for GH estimation was drawn at +60, +90, +120 and +180 minutes. The subjects remained in a supine position throughout the procedure.

The samples were immediately centrifuged and stored at -80° C until analysis. Analysis was held out in batches and blind to subjects status.

Assays

Growth hormone was measured in the Department of Biochemistry, Royal Infirmary, Glasgow using an "in house" immunoradiometric assay employing UK9 as primary working HGH standard. The sensitivity of the method is 0.2 mU/l (CV <22%) with a between batch variation of 5% over the working range 5 to 40 mU/l.

Statistical evaluation

Student's t-tests were employed to compare baseline growth hormone values in patient with CFS and healthy controls. The same tests were applied comparing Δ GH (difference between baseline values and the maximum increase post pyridostigmine administration) values in two groups.

Growth hormone responses over time were compared by repeated measures two-way ANOVA and Tukey's post hoc comparisons were applied. Results are expressed as mean \pm SEM.

3.11 STUDY 6: Growth hormone release in response to dopamine agonist - bromocriptine:

Subjects

24 subjects participated in this study, 12 healthy subjects and 12 patients with CFS. All patients and healthy subjects gave written consent for their participation. All patients fulfilled the strict criteria for CDC revised case definition. All had an extensive clinical work-up as in-patients to exclude all known causes of chronic fatigue. CFS group consisted of 6 males, mean age 39 years, and 6 females, mean age 37 years.

6 men and 6 women, both with mean age of 35 years, were included in the control subjects. None had a personal or family history of psychiatric disorder. All females were tested within the first ten days of menstrual cycle.

Procedure

All patients were hospitalised the night before the test. The test was commenced in the morning following an overnight fast. An intravenous cannula was inserted into a forearm vein at 8.30 am and they were allowed to relax for 30 minutes. Baseline blood for growth hormone estimation was drawn at 9.00 am. The cannula was kept patent by flushing it with heparinase (0.5 ml, 50 units) after samples of blood were taken and the first 2 mls extracted at each time point was discarded. Bromocriptine 2 mgs orally was administered at 0 minute, i.e. at 9.00 am and further blood for GH estimation was drawn at +60, +90, +120 and +180 minutes. The subjects remained in a supine position throughout the procedure.

The samples were immediately centrifuged and stored at -80° C until analysis. Analysis was held out in batches and blind to subjects status.

Assay

Growth hormone was measured in the Department of Biochemistry, Royal Infirmary, Glasgow using an "in house" immunoradiometric assay employing UK9 as primary working HGH standard. The sensitivity of the method is 0.2 mU/l (CV <22%) with a between batch variation of 5% over the working range 5 to 40 mU/l.

Data analysis

Results were expressed as mean \pm SEM and statistically analysed as follows.

Baseline GH and Δ GH (measured by subtracting the baseline from the peak value after bromocriptine) values were compared in patients with CFS and controls by using two-tailed student's t-tests. Two-way ANOVA with repeated measures was employed to compare GH response curves to bromocriptine in healthy subjects and patients with CFS.

3.12 STUDY 7: Baclofen induced growth hormone release:

Subjects

Twenty four subjects - twelve patients and twelve healthy controls - gave fully informed consent to participate in this study. The CFS group consisted of six men and six women between the ages of 33 and 53 years. All fulfilled the revised CDC criteria for chronic

fatigue syndrome. Healthy comparison group also consisted of six men and six women between the ages of 22 and 51 years. They had a full physical examination and psychiatric interview. None had a past or present history of chronic fatigue, psychiatric illness, neurological, endocrine, cardiovascular, renal or cerebrovascular disease. None had a history of heavy alcohol consumption or illicit drug. All females were tested in the follicular phase of their menstrual cycle.

Experimental procedure

The experiment was performed in the morning after an overnight fast. Between 8.30 am and 9.00 am a butterfly needle was inserted into an antecubital vein. Baseline blood samples were taken at 0 minute and thereafter at +60, +90, +120 and +180 minutes for the estimation of growth hormone (GH). All subjects received 20 mgs of baclofen orally at 0 minute after baseline blood samples were drawn. Samples were immediately centrifuged and stored at -80° C until analysed for GH.

Assay

Growth hormone was measured in the Department of Biochemistry, Royal Infirmary, Glasgow using an "in house" immunoradiometric assay employing UK9 as primary working HGH standard. The sensitivity of the method is 0.2 mU/l (CV <22%) with a between batch variation of 5% over the working range 5 to 40 mU/l.

Statistical data

GH responses were measured as the maximum level post baclofen relative to baseline (Δ GH). Two-tailed student's t-tests were used to compare baseline and Δ GH means. A

two-way repeated measures ANOVA with planned comparisons was used to compare the hormone responses over time.

RESULTS

4.1 STUDY 1

Results: The prolactin responses to buspirone challenge were considerably augmented in patients with CFS. The change in prolactin (Δ prolactin) level was taken as the difference between the baseline value and the maximum value after buspirone. There were no differences in basal prolactin levels between two groups: CFS 367.27 ± 21.17 mU/L; healthy controls 362.93 ± 23.86 mU/L, ($P = 0.8$). Δ Prolactin levels differed significantly between patients with chronic fatigue syndrome and controls (unpaired t-test: $t = 2.7$, $d.f = 58$, $P < .009$) (see fig. 4.1.1).

Using a repeated measures two-way ANOVA to compare responses over time between CFS and controls yields a significant group x time interaction ($F = 3.59$; $d.f = 4, 295$; $P = .007$). Tukey comparisons reveal differences ($P < 0.05$) between the groups at +60, and +120 minutes (see fig. 4.1.2).

Buspirone challenge (delta Prolactin)

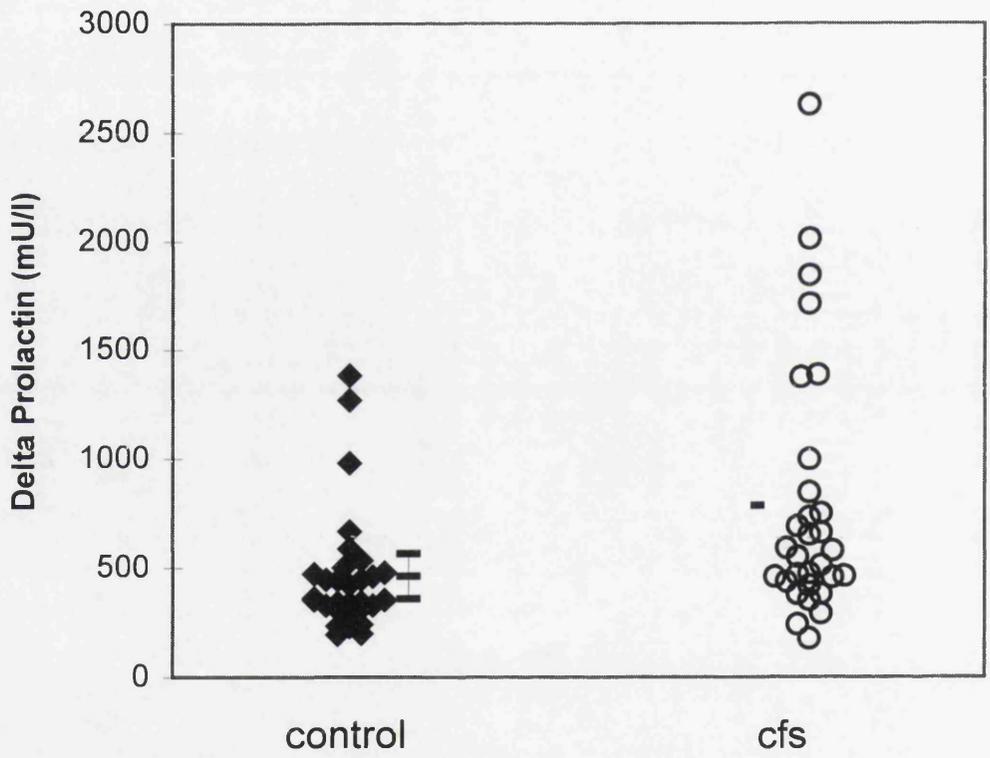


Figure 4.1.1

Prolactin (PRL) responses (Δ prolactin) to buspirone challenge in chronic fatigue syndrome (CFS) - O - and in healthy controls - \blacklozenge -. Results are expressed as the mean \pm SEM with 95% confidence limit in control subjects to compare with mean \pm SEM (-) of CFS group.

Buspirone induced Prolactin release

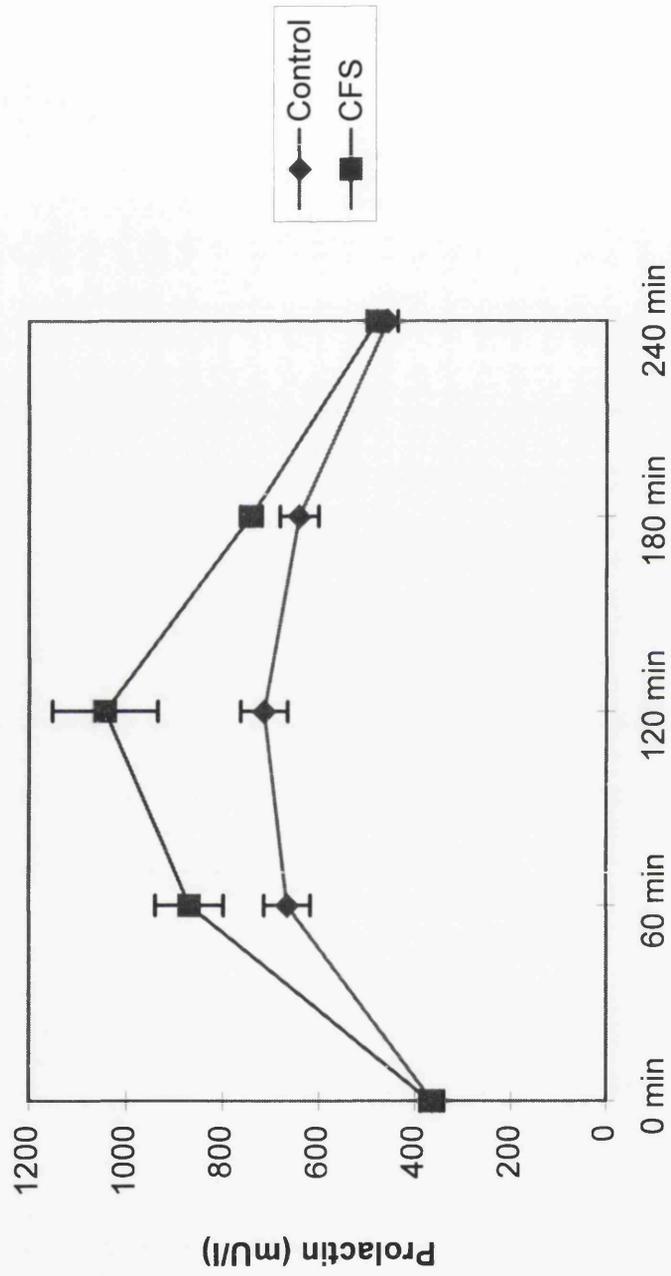


Figure 4.1.2

Mean plasma prolactin responses (\pm SEM) as a function of time after buspirone administration to 30 patients with CFS and matched controls. Post hoc Tukey following ANOVA was used to compare changes at different time points.

4.2 STUDY 2

Results: Baseline ACTH levels did not differ significantly between the two groups: CFS 18.6 ± 2.3 ng/L, healthy controls 20.7 ± 1.9 ng/L; $t = 0.74$, $d.f = 27$, $P = 0.46$ (see fig. 4.2.1). Neither did baseline cortisol levels differ significantly: CFS 433.5 ± 49.3 nmol/L, healthy subjects 453.7 ± 65.1 nmol/L (see fig. 4.2.2).

Thirteen of the fourteen healthy volunteers elevated ACTH in response to ipsapirone challenge. Eight of the CFS patients failed to show a response. The mean \pm SEM Δ ACTH values for both groups were: CFS 4.4 ± 0.6 ng/L, healthy subjects 14.6 ± 1.6 ng/L. Overall CFS patients release less ACTH than do the healthy comparison subjects ($t = 5.9$, $d.f = 27$, $P < 0.01$) (see Figure 4.2.3). A repeated two-way ANOVA yields a significant group \times time interaction ($F = 2.4$; $d.f = 5, 155$; $P = 0.038$). Tukey comparisons reveal differences ($P < 0.05$) between the groups at +60, +90 and +120 minutes (see fig. 4.2.4). Cortisol responses to ipsapirone challenge were inconsistent with only 6 healthy subjects and 5 chronic fatigue patients showing clear increases from baseline. The overall mean \pm SEM response was 50.6 ± 58.4 nmol/L in CFS and 39.2 ± 60.3 nmol/L in healthy subjects. These differences were not significant.

Overall ipsapirone was well tolerated, though mild nausea and lightheadedness was reported by subjects in both groups.

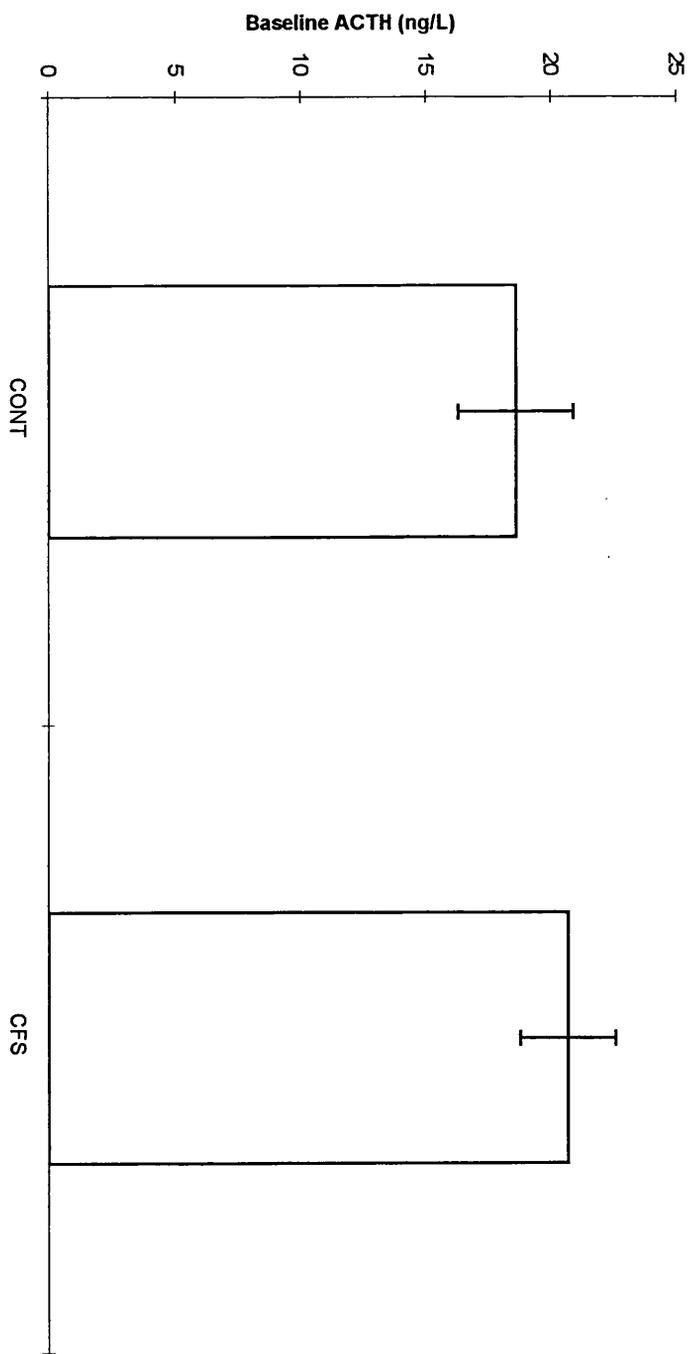


Figure 4.2.1

Baseline ACTH levels in patients with chronic fatigue syndrome (CFS) (n=14) and in healthy comparison subjects (CONT) (n=14). Results are expressed as the mean \pm SEM.

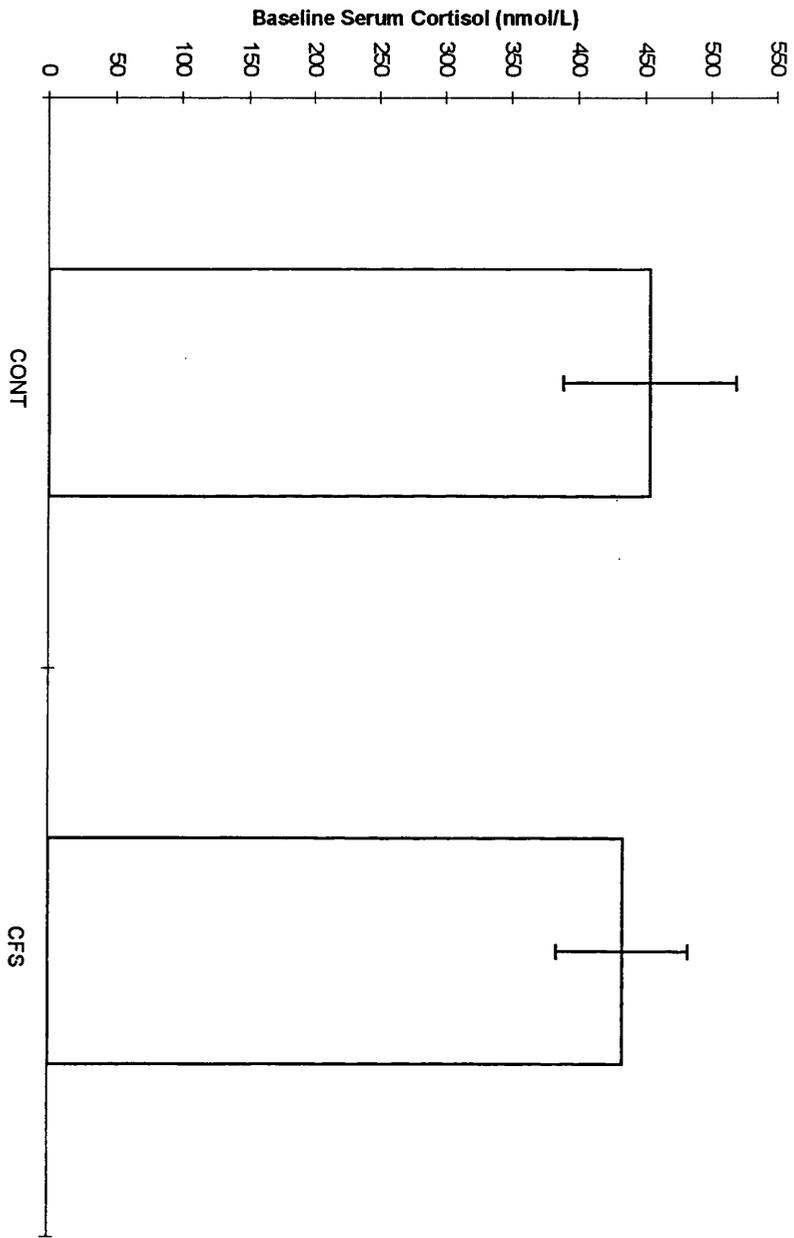


Figure 4.2.2

Baseline cortisol levels in patients with chronic fatigue syndrome (CFS) (n=14) and in healthy comparison subjects (CONT) (n=14). The results are expressed as the mean \pm SEM.

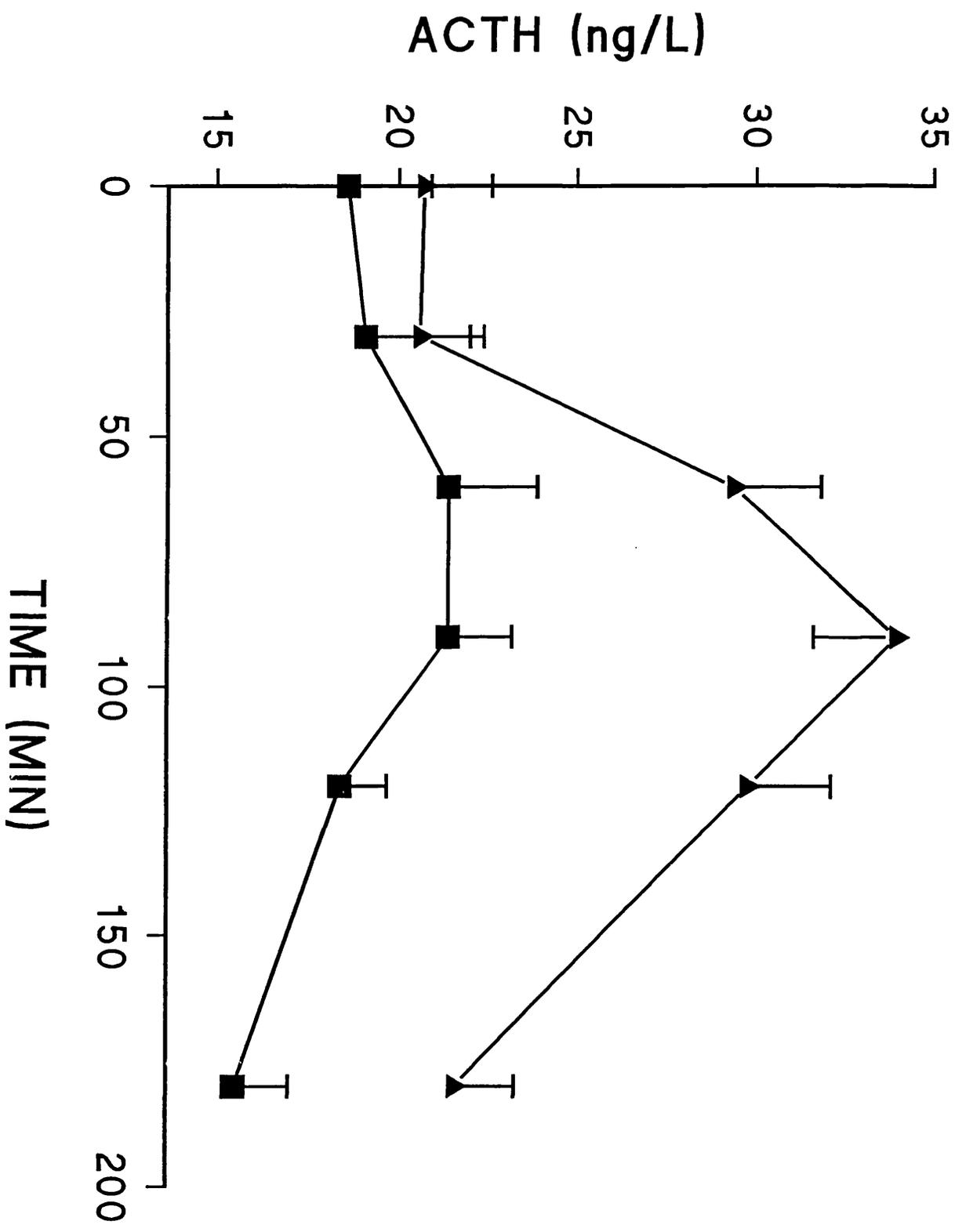


Figure 4.2.3

ACTH responses to ipsapirone challenge in chronic fatigue syndrome (CFS) (-) and in healthy comparison subjects (◆). Ipsapirone (20 mg orally) was administered at time 0 minute. Results are expressed as the mean \pm SEM.

Δ ACTH (ng/L)

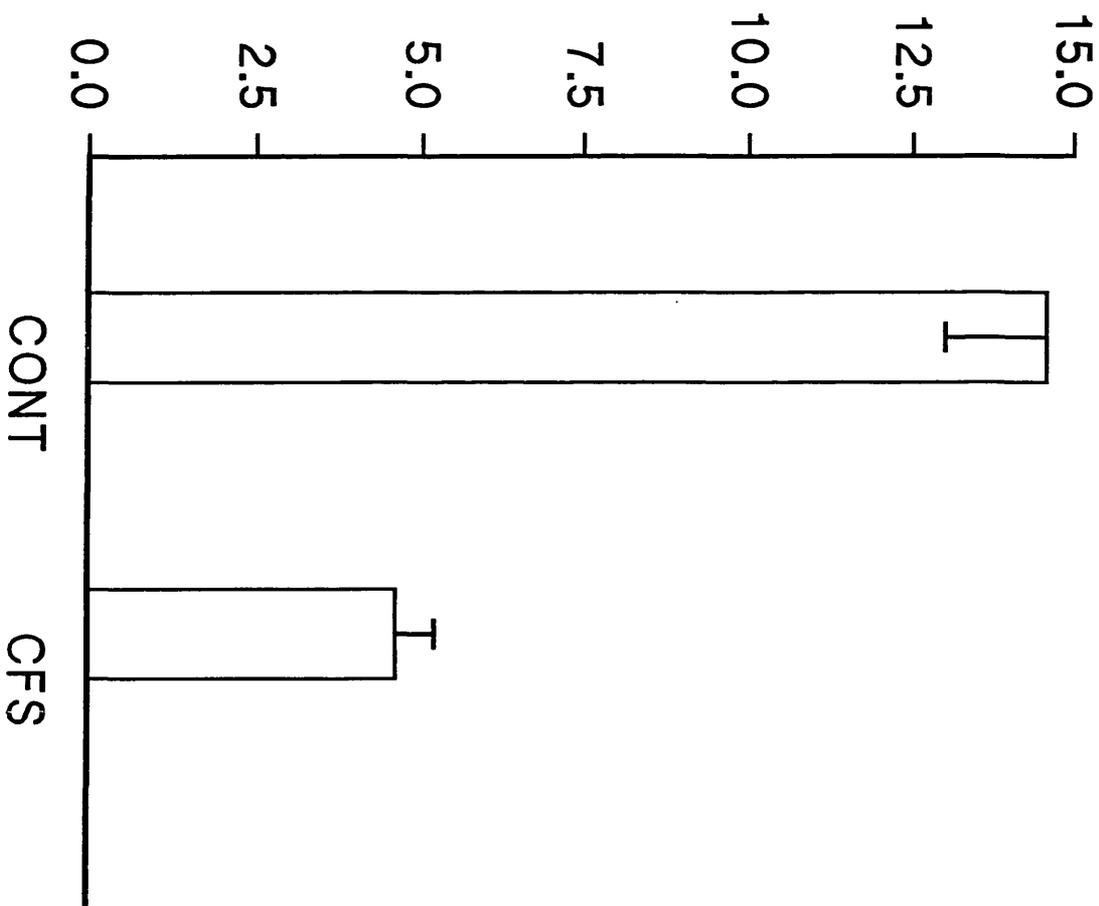


Figure 4.2.4

ACTH responses (Δ ACTH) to ipsapirone challenge in chronic fatigue syndrome (CFS) and in healthy comparison subjects (CONT). Results are expressed as the mean \pm SEM.

4.3 STUDY 3

Results: Using one-way ANOVA baseline GH values (control group 1.4 ± 0.31 mU/L; CFS 0.73 ± 0.16 mU/L; depressives 1.07 ± 0.25 mU/L) did not differ significantly between the three groups [F (2,33) = 2.23, P = 0.123].

The overall growth hormone levels rose in response to dexamethasone (DEX) challenge. Growth hormone responses to dexamethasone (Δ GH) were calculated as the maximum GH level post-DEX relative to baseline. In the first phase Δ GH values (control 19.17 ± 3.8 mU/L; CFS 4.8 ± 1.52 mU/L; depressives 3.44 ± 1.07 mU/L) were significantly different [F (2,33) = 29.34; P < .0001] but CFS and depressives were not significantly different from each other (see fig. 4.3.1).

Metyrapone treatment resulted in a nearly significant drop in cortisol in all three groups. In depressives it dropped from 820.9 ± 159.5 nmol/L to 355.6 ± 101.2 nmol/L; in healthy controls from 453.7 ± 65.1 nmol/L to 298.7 ± 87.6 nmol/L and CFS from 501.4 ± 102.26 nmol/L to 342.4 ± 98.4 nmol/L (see fig. 4.3.5).

Δ GH values after metyrapone treatment in three groups (control 26.98 ± 4.12 mU/L; CFS 6.33 ± 2.03 mU/L; depressives 4.33 ± 1.03 mU/L) were different significantly [F (2,33) = 17.65, P < 0.0001] (see fig. 4.3.3). Of the three groups healthy volunteers increased Δ GH from 19.17 ± 3.8 mU/L to 26.98 ± 4.12 mU/L (P = 0.09) after dexamethasone following metyrapone treatment compared to CFS and depressives (P = 0.5).

Tukey comparisons following repeated measures two-way ANOVA to compare responses over time between controls, CFS and depressives reveal differences (P < 0.05)

at 60, 120 and 180 minutes in phase 1 and at 120, 180 and 240 minutes in phase 2 (see fig. 4.3.2 & 4.3.4).

DEX challenge (delta GH)

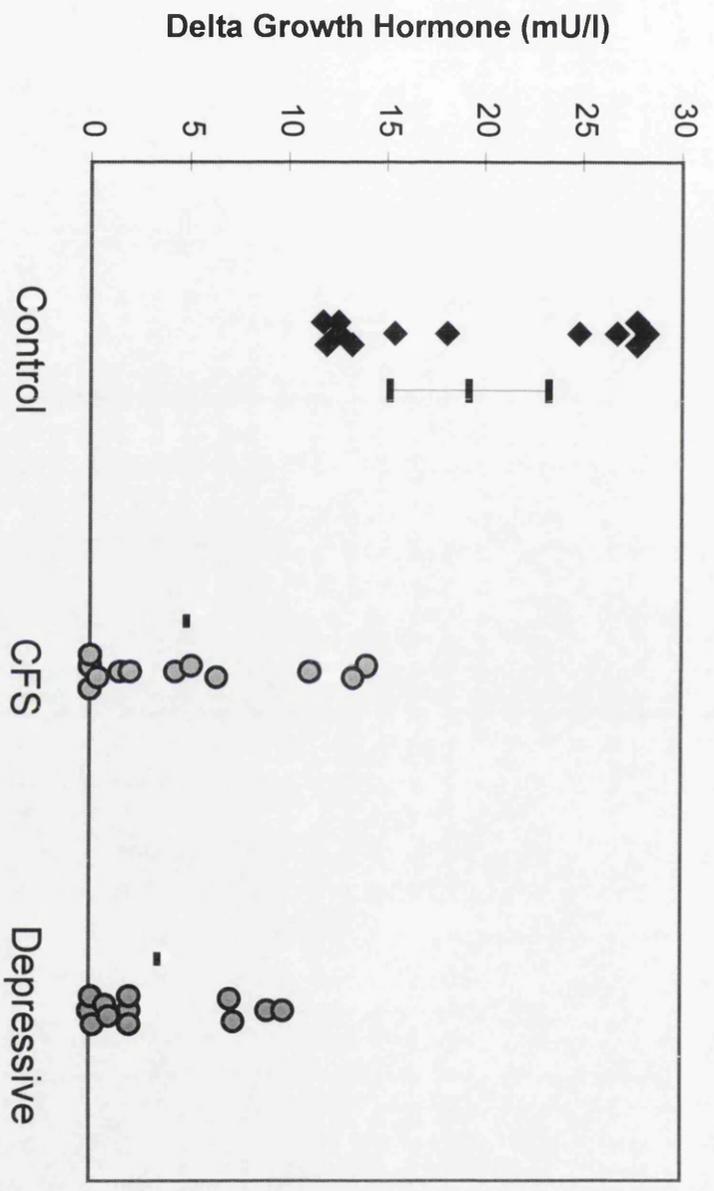


Figure 4.3.1

Phase 1. Growth hormone responses (Δ GH) to dexamethasone (DEX) in controls, patients with chronic fatigue syndrome (CFS) and in patients with depression. Results are expressed as mean \pm SEM with 95% confidence limit in controls to compare with mean \pm SEM of patients with chronic fatigue syndrome and depressives.

DEX induced GH release

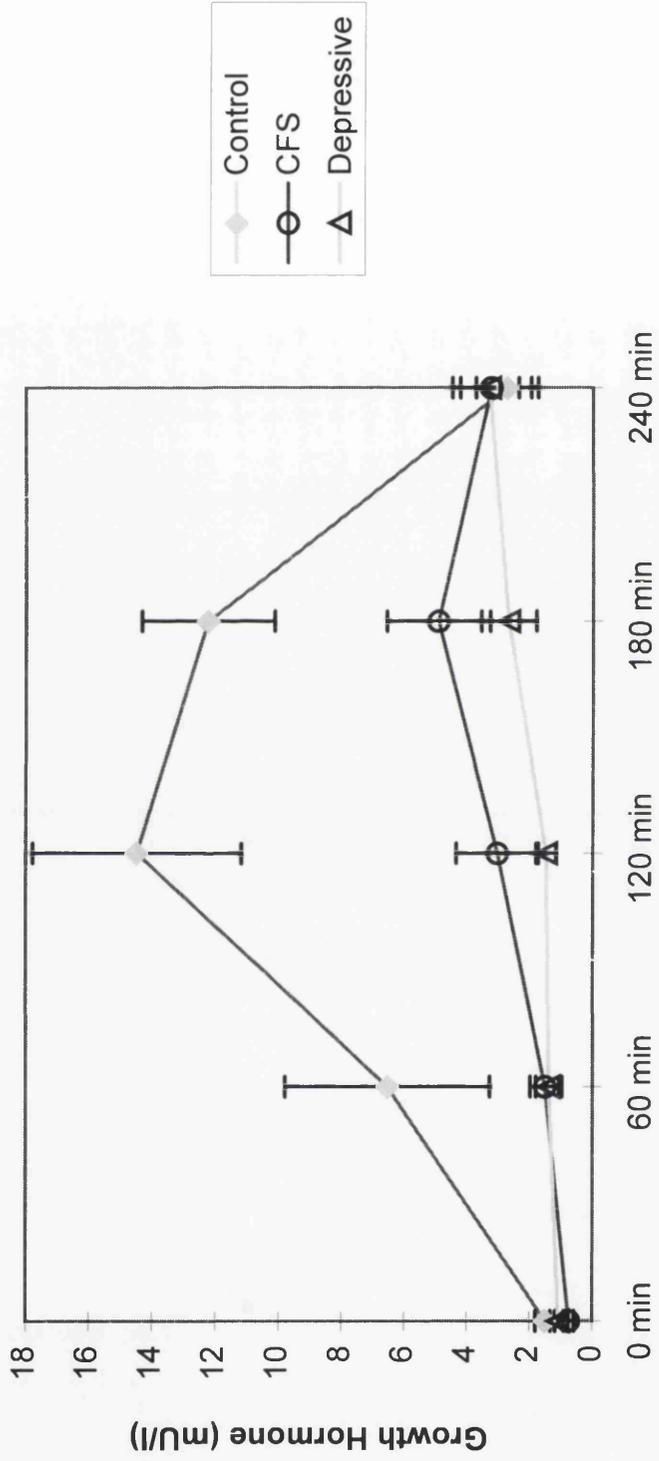


Figure 4.3.2

Phase 1. Mean plasma GH responses (\pm SE) as a function of time after DEX to patients with chronic fatigue syndrome, depressives and matched controls. Results are expressed as the mean \pm SEM.

Phase II - DEX challenge (delta GH)

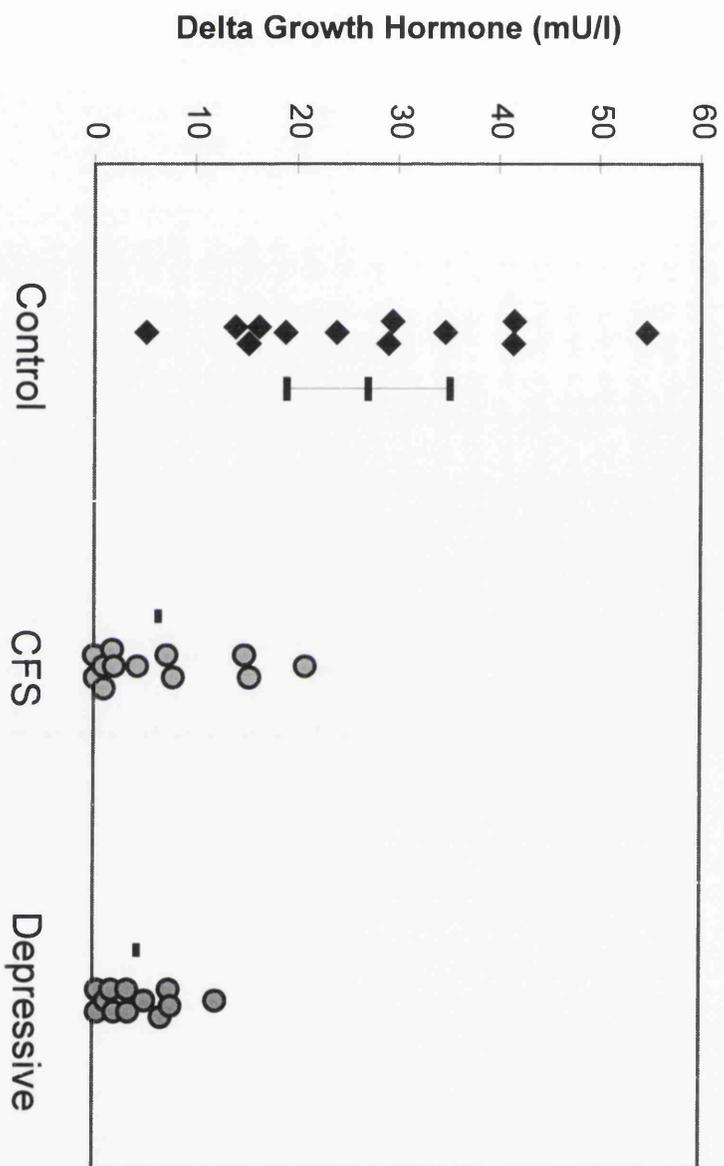


Figure 4.3.3

Phase 2. Growth hormone responses (Δ GH) to dexamethasone (DEX) in controls, patients with chronic fatigue syndrome (CFS) and in patients with depression. Results are expressed as mean \pm SEM with 95% confidence limit in controls to compare with mean \pm SEM of patients with chronic fatigue syndrome and depressives.

Phase II - DEX induced GH release

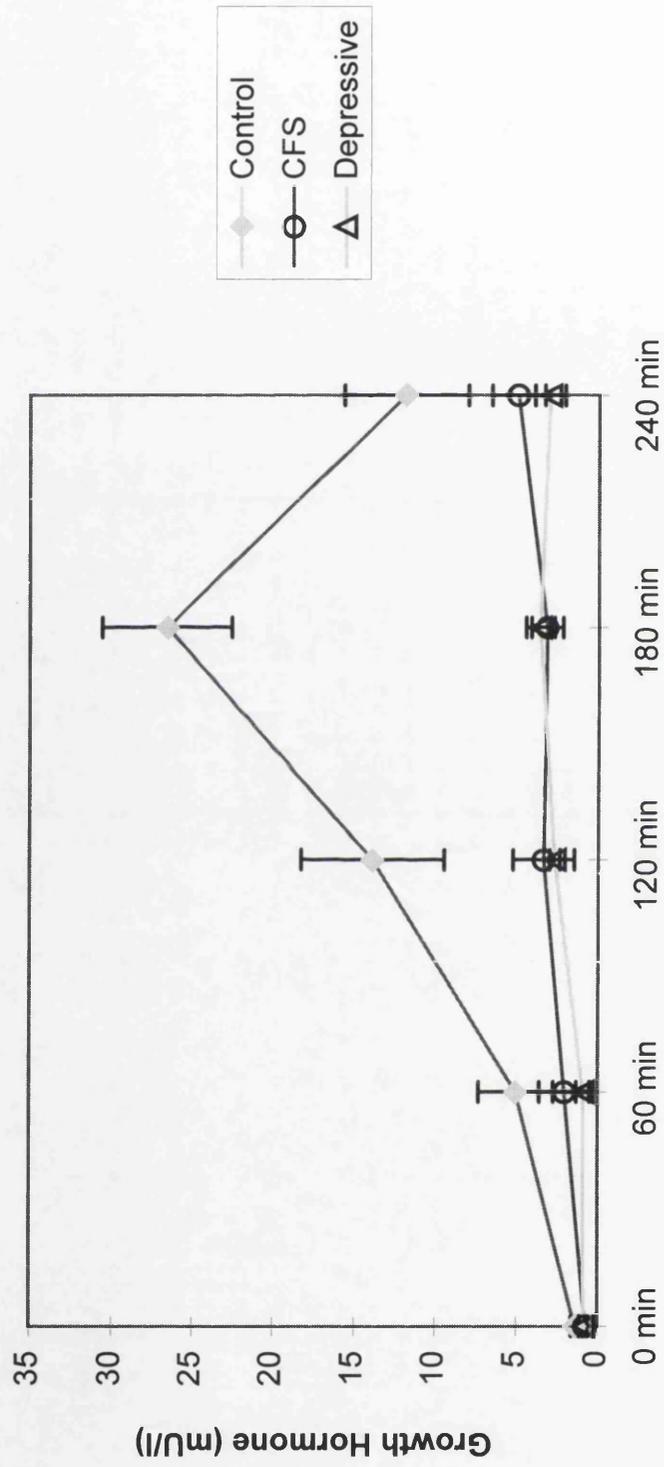


Figure 4.3.4

Phase 2. Mean plasma GH responses (\pm SE) as a function of time after DEX to patients with chronic fatigue syndrome, depressives and matched controls. Results are expressed as the mean \pm SEM.

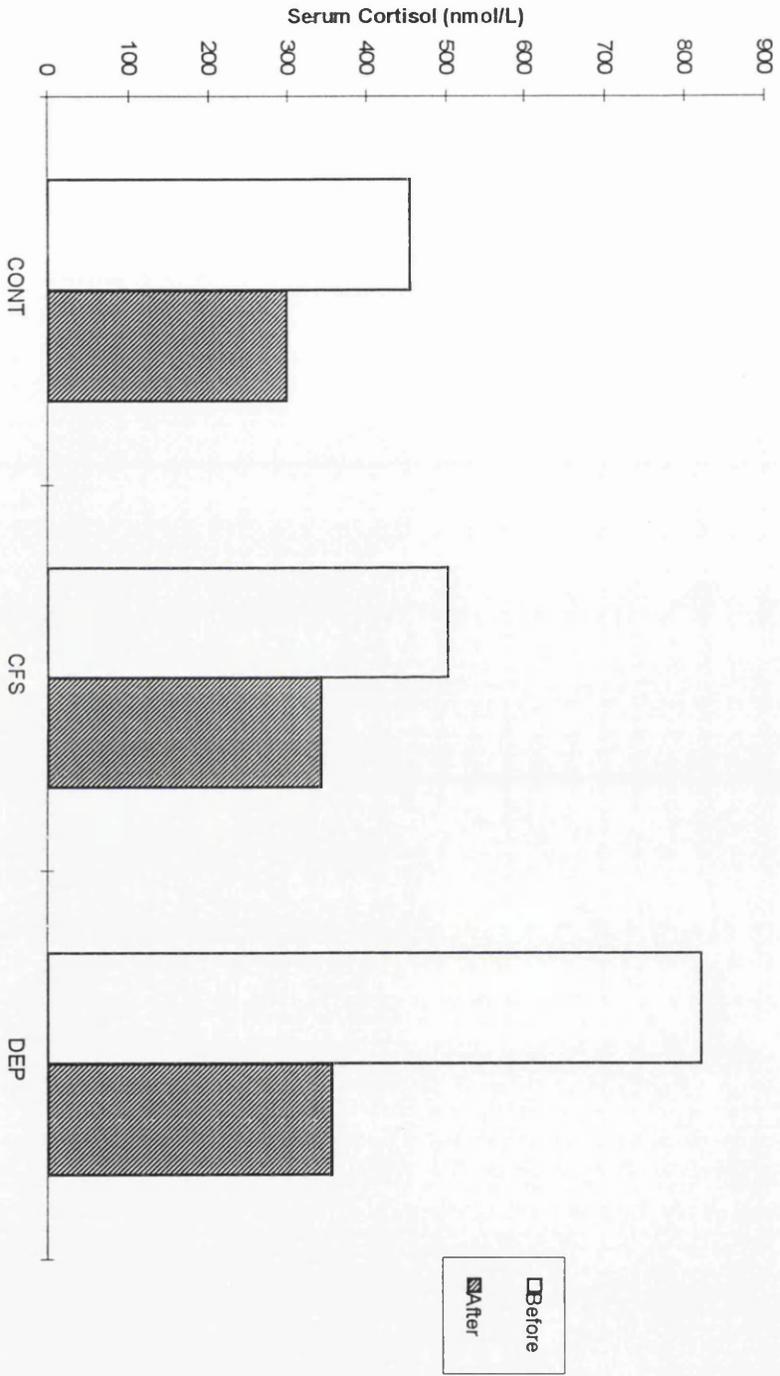


Figure 4.3.5

Mean cortisol levels in patients with chronic fatigue syndrome (CFS), depressives and healthy comparison subjects (CONT) before and after metyrapone.

4.4 STUDY 4

Results: Baseline GH levels did not differ significantly between the two groups; CFS 1.82 ± 0.15 mU/L, healthy controls 2.04 ± 0.2 mU/L; $t = 0.22$, d.f = 26, $P = 0.73$.

All healthy volunteers elevated GH in response to desipramine. Two of the chronic fatigue patients failed to show a response. A mean Δ GH in CFS group of 3.26 ± 0.51 mU/L was significantly lower compared to a mean Δ GH of 7.33 ± 1.38 mU/L in healthy controls ($t = 2.8$, d.f = 26, $P < 0.013$) (see fig. 4.4.1).

A repeated measures two-way ANOVA used to assess differences over time between CFS and healthy controls demonstrates a significant effect for group [$F(1,138) = 18.165$, $P = .0001$]; time [$F(4,135) = 14.98$, $P = .0001$] and the group x time interaction [$F(4,135) = 4.4$, $P = 0.002$]. Post-hoc Tukey comparisons show these responses to be significantly different at +120 ($P < 0.05$) and +180 minutes ($P < 0.05$) (see fig. 4.4.2).

Desipramine challenge (delta GH)

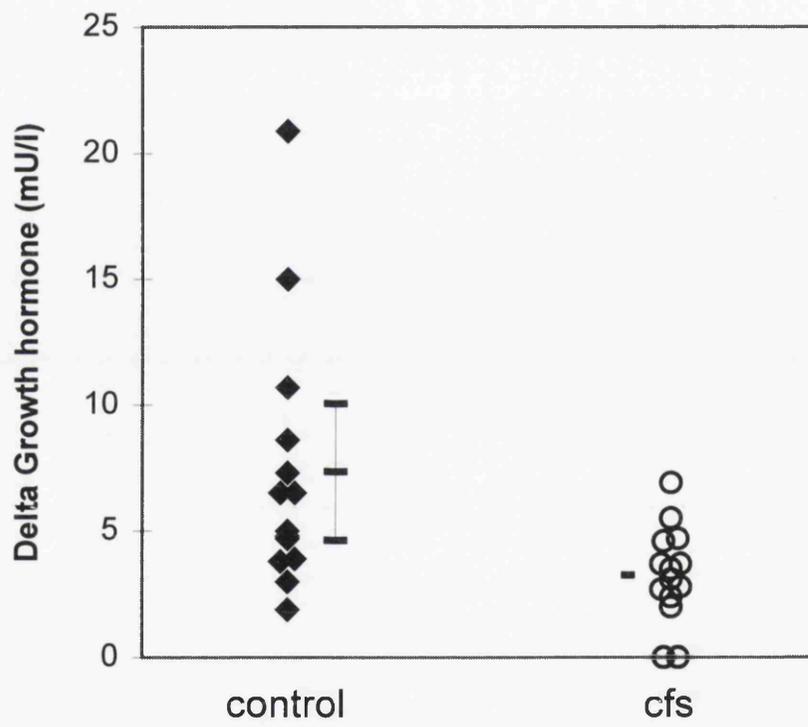


Figure 4.4.1

Growth hormone responses (Δ GH) to desipramine in controls -♦- and patients with chronic fatigue syndrome (CFS) -O-. The results are expressed as the mean \pm SEM with 95% confidence limit in controls to compare with mean \pm SEM of CFS group.

Desipramine induced GH release

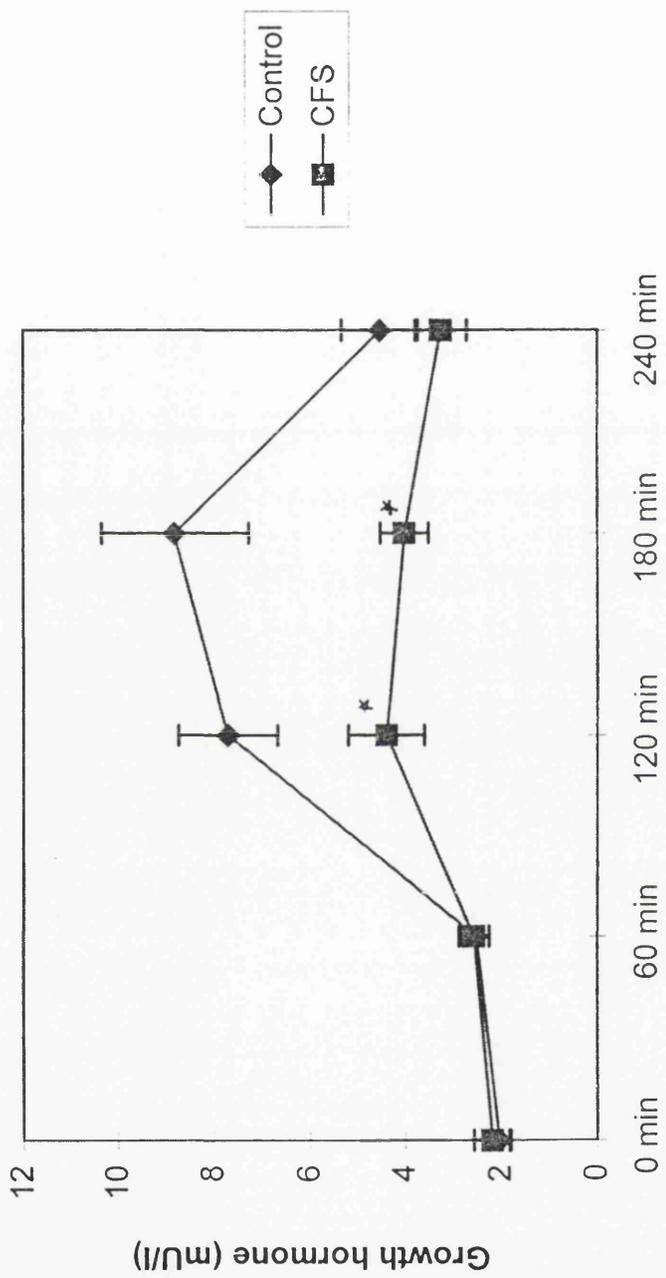


Figure 4.4.2

Growth hormone responses (mean \pm SEM) as a function of time after desipramine challenge in patients with CFS and matched controls.

4.5 STUDY 5

Results: All but one healthy individual responded to pyridostigmine with an increase in plasma growth hormone. There were no differences in baseline GH levels between CFS and control groups (CFS 0.63 ± 0.28 mU/L, control 0.71 ± 0.11 mU/L; $t = 0.22$, d.f = 26, $P = 0.78$).

A mean Δ GH in CFS group of 17.07 ± 2.57 mU/L was significantly higher compared to a mean Δ GH of 6.84 ± 1.26 in healthy controls ($t = 3.5$, d.f = 26, $P < 0.002$) (see fig. 4.5.1).

Using a repeated measures two-way ANOVA to compare responses over time between CFS and controls yields significant effect for group [$F(1,138) = 14.178$, $P = .0003$] and time [$F(4,135) = 8.84$, $P = .0001$]. Tukey post-hoc analysis indicates that GH responses at +60, +120 and +180 minutes are significantly different ($P < 0.05$) (see fig. 4.5.2).

Pyridostigmine challenge (delta GH)

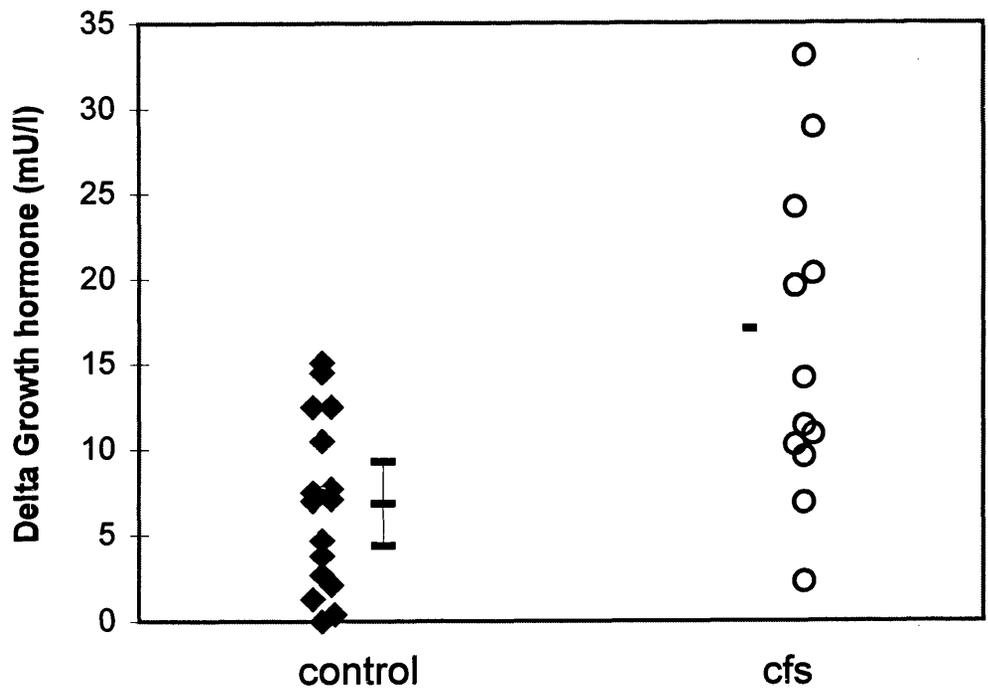


Figure 4.5.1

Growth hormone responses (Δ GH) to pyridostigmine in controls -♦- and patients with chronic fatigue syndrome (CFS) -O-. Results are expressed as the mean \pm SEM with 95% confidence limit in controls to compare with mean \pm SEM of CFS group.

Pyridostigmine induced GH release

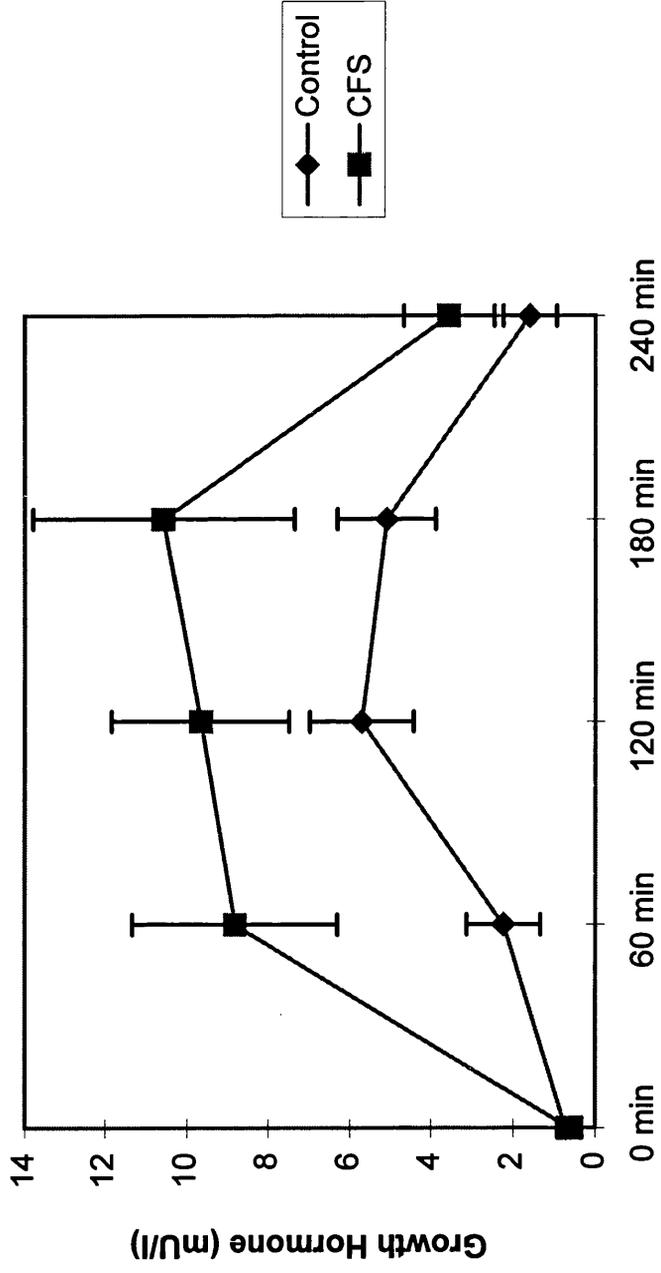


Figure 4.5.2

Plasma growth hormone responses (mean \pm SEM) as a function of time after pyridostigmine in patients with chronic fatigue syndrome (CFS) and matched controls. Post-hoc Tukey comparisons were used to compare changes over time in both groups.

4.6 STUDY 6

Results: The overall GH levels rose in response to bromocriptine in both healthy subjects and patients with CFS. In fact, two-way ANOVA with repeated measures disclosed no significant differences between the two groups [$F = 0.080$, NS] and no significant group x time interaction ($F = 0.308$, NS) but it showed a significant effect for time ($F = 12.44$, $P = .0001$). Tukey's post-hoc analysis indicates that responses at +60, +120, +180 and +240 minutes are not different in both groups (see fig. 4.6.1).

No significant difference in mean Δ growth hormone (Peak GH after bromocriptine - baseline GH) is seen in CFS (8.93 ± 1.32 mU/L) compared with the control group (8.53 ± 1.95 mU/L) [unpaired t-test: $t = 0.1$, d.f = 22, $P = 0.86$] (see Figure 4.6.2).

Bromocriptin induced GH release

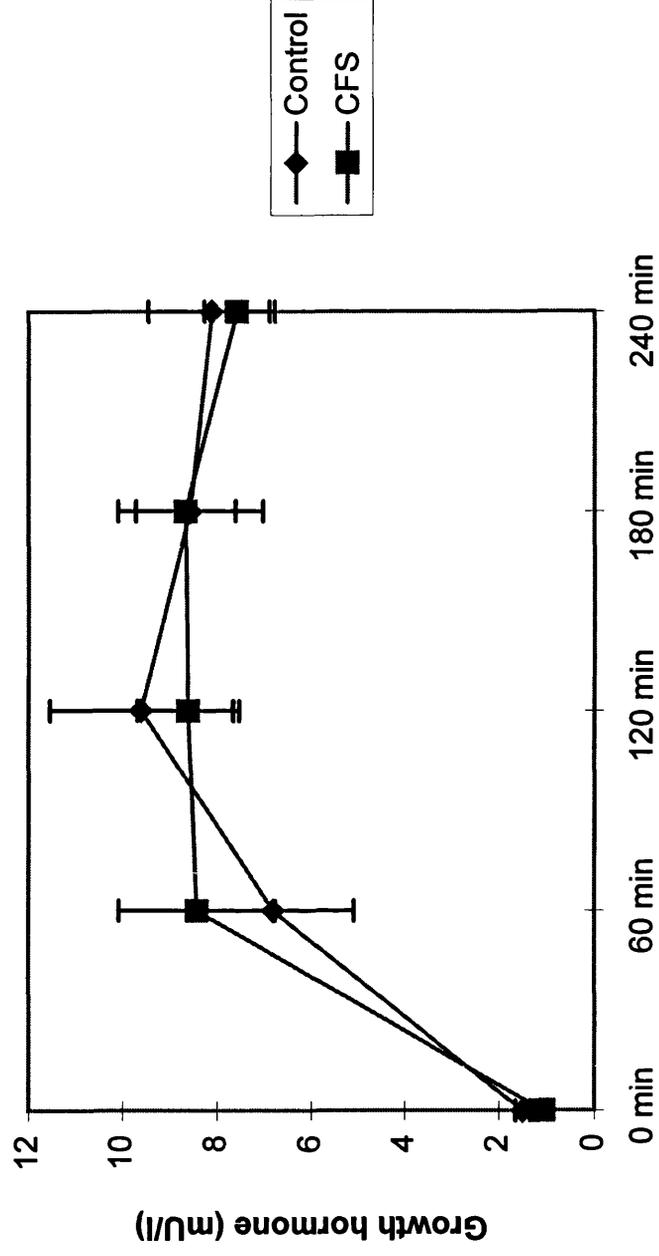


Figure 4.6.1

Plasma growth hormone responses (mean \pm SEM) as a function of time after bromocriptine (2 mg orally) to patients with CFS and matched controls. Post-Tukey following ANOVA compared changes after bromocriptine in controls and patients.

Bromocriptin challenge (delta GH)

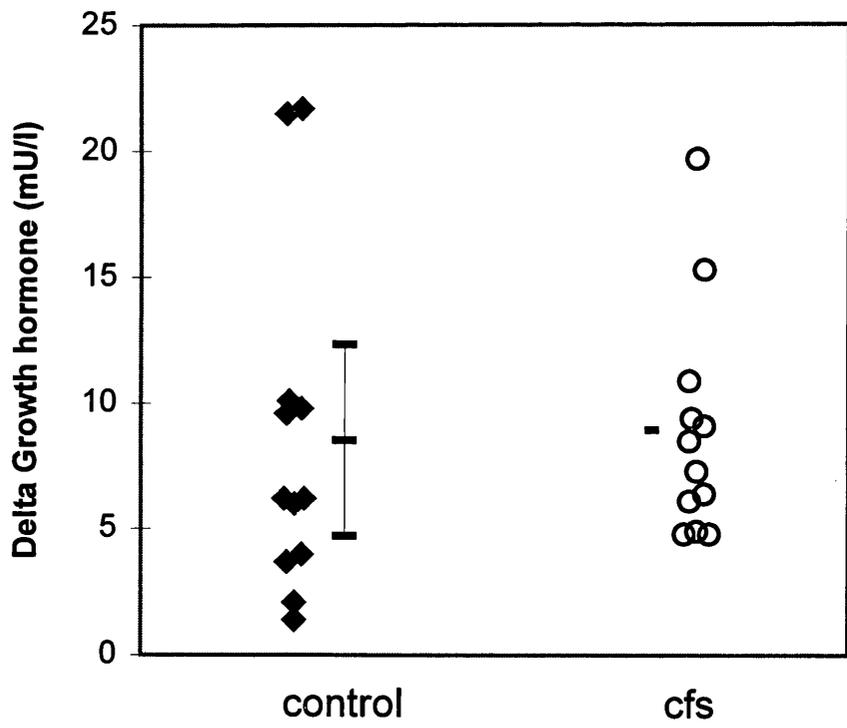


Figure 4.6.2

Growth hormone responses (Δ GH) to bromocriptine in controls -♦- and patients with chronic fatigue syndrome (CFS) -O-. Results are expressed as mean \pm SEM with 95% confidence limit in controls to compare with mean \pm SEM of CFS group.

4.7 STUDY 7

Results: All patients and healthy volunteers responded to baclofen with an increasing plasma GH. There were no differences in basal GH levels between two groups (unpaired t-test: $t = 0.1$, $d.f = 22$, $P = 0.80$). Similarly no significant difference in Δ GH (Peak GH post-baclofen - baseline GH) is seen in CFS compared to controls [CFS 12.70 ± 2.18 mU/L, control 11.05 ± 1.98 mU/L, unpaired t-test: $t = 0.686$, $d.f = 22$, $P = 0.58$) (see fig. 4.7.1).

ANOVA with repeated measures disclosed no significant difference between two groups ($F = 0.060$, NS) and no significant group x time interaction ($F = 0.26$, NS) but it showed a significant effect for time ($F = 14.28$, $P < .0001$). Tukey post-hoc analysis indicates that responses at +30, +60, +90, +120 and +180 minutes are not different in both groups (see fig 4.7.2).

Baclofen challenge (delta GH)

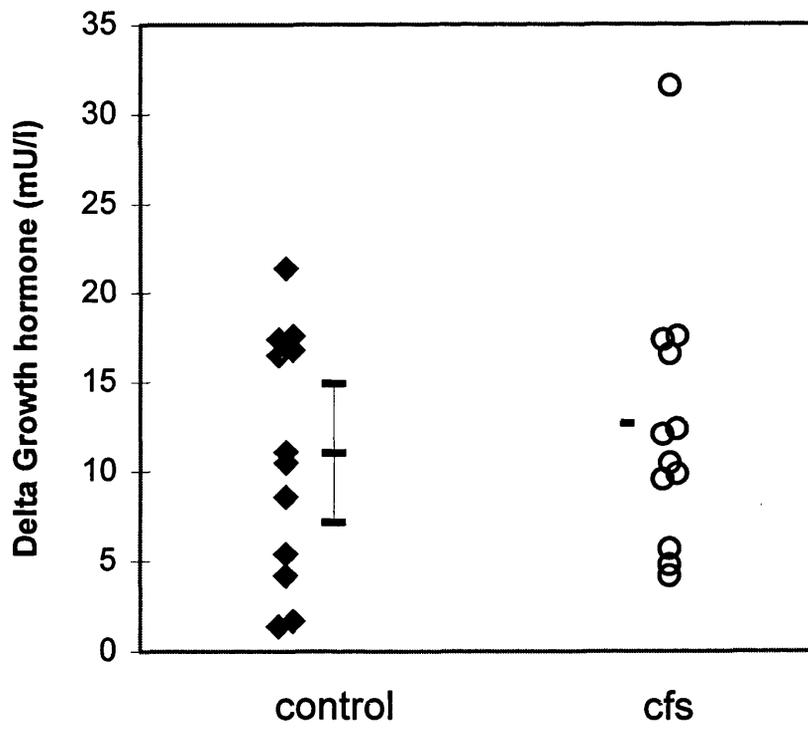


Figure 4.7.1

Growth hormone responses (Δ GH) to baclofen in controls -♦- and patients with chronic fatigue syndrome (CFS) -O-. Results are expressed as mean \pm SEM with 95% confidence limit in controls to compare with mean \pm SEM of CFS group.

Baclofen induced GH release

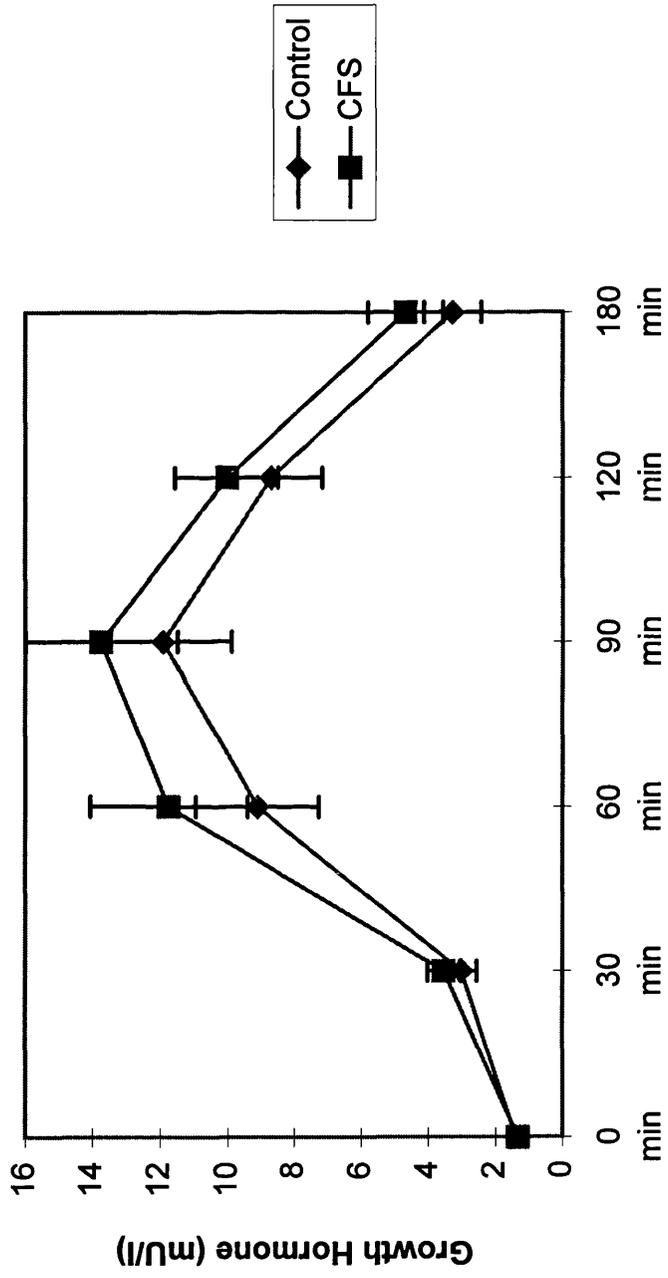


Figure 4.7.2

Growth hormone responses (mean \pm SEM) as a function of time after baclofen (20 mg orally) to patients with CFS and matched controls. Post-Tukey following ANOVA was used to compare changes over time after baclofen challenge in both groups.

DISCUSSION AND CONCLUSION

DISCUSSION

Chronic fatigue syndrome is an important disease posing enormous health problems that urgently need elucidation. Research has been hampered in this illness by the fact that there is no diagnostic test and the present definitions mentioned earlier in this thesis have not been specific. The definition which I used in the studies of this thesis met the CDC (Holmes et al 1988; Fukuda et al 1994) , UK (Sharpe et al 1991) and Australian (Lloyd et al 1990) criteria for defining this illness. In addition, as stated in methods, all the patients were hospitalised as in-patients and fully investigated to exclude any medical or psychiatric causes of their fatigue.

Analysis of the reported clinical symptoms and signs i.e. debilitating fatigue, mood and sleep disturbances, subjective feeling of hot and cold, emotional lability and temperature fluctuations - has strongly suggested that central/hypothalamic mechanisms are involved. Other reported data on chronic fatigue has further suggested hypothalamic involvement. Poteliakhoff (1981) found lower levels of plasma cortisol in patients suffering from chronic fatigue and suggested this to be due to decreased stimulation of the pituitary - adrenal axis secondary to exhaustion or malfunctioning of brain cells in the hypothalamic region. Asthenia and tiredness in post-hepatitis syndrome has also been attributed to impaired steroid metabolism (Mims 1969).

Initial studies in patients with CFS of impaired water metabolism (Bakheit et al 1993), augmented prolactin responses to 5HT1A agonist buspirone (Bakheit et al 1992) and abnormal adrenal cortisol sensitivity to exogenous ACTH and CRH with blunted ACTH responses to CRH (Demitrack et al 1991) has pointed to abnormalities of the hypothalamus and its activation of the pituitary - adrenal axis. Activation of the hypothalamic - pituitary - adrenal axis is brought about by the release of CRH from the paraventricular nucleus which is under the control of a wide array of neurotransmitters.

For this reason we decided to analyse the regulation of different neurotransmitter systems and their influence on the functional activity of hypothalamic - pituitary - adrenal (HPA) axis, hypothalamic somatotroph axis and hypothalamic prolactin axis.

Dynamic neuroendocrine challenge tests provide one of the only methods for examining neurotransmitter function *in vivo* in humans (Checkley 1980). This neuroendocrine approach is heavily dependent on the development of selective drugs for challenging specific receptor systems. The resultant hormonal response to drug challenge provide an important bio-assay for the evaluation of neurotransmitter function - integrity of it's pathway and the sensitivity of it's receptor systems. The various neurotransmitter systems which were studied in this thesis include dopamine (DA), noradrenaline (NA), 5-hydroxytryptamine (5-HT), gamma aminobutyricacid (GABA) and acetylcholine (ACh).

5-HT mediated prolactin release:

In the first study we extended the original work of Bakheit et al (1992) on buspirone induced prolactin (PRL) release. Our data confirmed augmented PRL release in response to buspirone in patients with CFS compared with healthy controls. We did not look at depressives in this study but our data confirmed that there was an increased response as compared to the blunted response reported in patients with depression (Bakheit et al 1992).

In general prolactin secretion from the anterior pituitary is likely to be mediated by many different pathways but is primarily under tonic dopaminergic (DA) inhibitory control (Kato et al 1985). Dopamine is released from the terminals of the tuberoinfundibular dopaminergic neurones into the pituitary portal circulation (MacLeod 1976; Gudelsky

and Porter 1979) and exerts its inhibitory effect on PRL secretion via DA₂ receptors located on pituitary lactotrophs. Consequently dopamine agonist inhibit the release of prolactin (Meltzer and Fang 1976) and neuroleptics that block the DA receptors (dopamine antagonists) augment prolactin release (Meltzer et al 1978). In contrast to dopamine, the effect mediated by serotonin (5HT) is stimulatory and indirect as there are no 5HT receptors on the lactotrophs (Lambarts and MacLeod 1978).

It is generally accepted that serotonergic neurons in the dorsal raphe nucleus stimulate the secretion of prolactin (Advis et al 1979, Barofsky et al 1983, Kitts and Johnson 1986, Van De Kar and Bethea 1982, Van De Kar et al 1985) probably through secretion of prolactin releasing factor (PRF) in the paraventricular nucleus of the hypothalamus (Minamitani et al 1987). Whether 5-HT can also release prolactin by a direct action on the pituitary gland is at present controversial (Stobie and Shin 1983; Lopez et al 1987). A large number of different 5-HT receptors have been discovered on neurons in the human brain. These include 5-HT_{1A}, 5-HT_{1D}, 5-HT_{1E}, 5-HT_{1F}, 5-HT_{2A}, 5-HT_{2C} (previously called 5-HT_{1C} receptor). The role of different 5-HT receptor subtypes in stimulating the secretion of prolactin are less clear. Some studies indicate that prolactin response to 5-HT stimulation is mediated predominantly by 5-HT₁ rather than 5-HT₂ receptors (Charig et al 1986) whereas Jahn and Deis (1988) report some evidence that there is stimulatory action of serotonin on prolactin release by 5-HT₂ receptors. In rats evidence exists that 5-HT_{1B} receptors are involved in the stimulation of the secretion of prolactin by endogenously released 5-HT (Van De Kar 1989). As the human brain lacks 5-HT_{1B} recognition sites (Hoyer et al 1986) it can be concluded that the regulation of prolactin secretion in humans is mediated by 5-HT_{1A} or 5-HT₂ receptors or as yet undefined 5-HT receptor subtypes.

The exact mechanisms of buspirone induced prolactin release in humans and animals are unknown but several pieces of evidence suggest that on balance, prolactin release is mediated by 1. 5-HT receptors, 2. dopamine receptors, 3. non-specific stress factors such as induction of nausea. The latter is highly unlikely as none of our subjects reported any feelings of nausea. Animal studies indicate that buspirone produce a dose dependent increase in rat plasma prolactin levels (Meltzer et al 1982) through central dopaminergic mechanisms. A number of studies indicate that buspirone interacts with central dopaminergic mechanisms, e.g. it disrupts the conditioned avoidance response (Wu et al 1972; Allen et al 1974) and blocks apomorphine induced stereotypy in rats and emesis in dogs (Allen et al 1974; Stanton et al 1981). Radiolabelled ligand binding studies also indicate that buspirone can interact with dopamine (DA) receptors (Stanton et al 1981). It was also proposed that, in low doses, it may also act as dopamine agonist (Taylor et al 1980, Riblet et al 1981) however, enhancement, rather than a reversal by buspirone of alpha-methyl-Para-tyrosine (AMPT) induced increase in prolactin secretion strongly argues against the hypothesis that buspirone acts as a dopamine agonist at the pituitary gland (Meltzer et al 1982).

If this increase in prolactin release is mediated through DA receptors it can be hypothesised that dopaminergic inhibitory tone on anterior pituitary lactotrophs for the release of prolactin is higher in patients with CFS compared with controls. This postulated altered DA neurotransmitter function in CFS was further tested by giving the patients dopamine agonist - bromocriptine and measuring growth hormone release, no difference was found between patients and controls. It is therefore unlikely that the increase in prolactin release in response to buspirone in patients with CFS is through dopaminergic receptors.

The involvement of 5-HT receptors in buspirone induced prolactin release is supported by the observation that 5-HT antagonist - methysergide (Kato et al 1974) and metergoline (Gregory et al 1990) significantly reduced prolactin release in response to buspirone.

Finally, it is concluded that prolactin release in response to buspirone is mediated through 5-HT_{1a} receptors in the hypothalamus and an augmented response suggest upregulation/increased sensitivity of central 5-hydroxytryptamine receptors in CFS. Other studies which support the idea of increased sensitivity of central 5-HT receptors in this illness are those of Cleare et al (1995). They found similar augmentation in prolactin release when 5-HT- releasing agent d-fenfluramine was used. It assesses "net" 5-HT activity as it is not clear which 5-HT receptor subtypes are involved in mediating these responses. Demitrack et al (1992) in their study also provide evidence of disturbed 5-HT functioning in the periphery. In 19 patients with CFS they found increased plasma 5-hydroxyindole acetic acid (HIAA) the major metabolite of 5-HT. An increase in peripheral turnover might explain the heightened allergic responsiveness, as well as the musculoskeletal pain seen in these patients, whilst altered central 5-HT may explain the cognitive fatigue, poor concentration and sleep disturbance.

As stated in the method section, we tested pre-menopausal women in the early follicular phase of menstrual cycle. Dinan et al (1990) reported that prolactin responses to buspirone vary widely throughout the menstrual cycle in healthy women with a three fold increase pre-menstrually probably through upregulation of 5HT receptors following ovulation when gonadal steroid levels are high (Biegon et al 1980 and Ladisich 1977).

The view that chronic fatigue syndrome is a form of depressive disorder is not supported by the findings of this study. In depression, prolactin responses to 5HT_{1A} agonist

bupirone (Bakheit et al 1992), 5-HT reuptake inhibitor clomipramine (Anderson et al 1992) and to d-fenfluramine (Cleare et al 1995, O'Keane and Dinan 1991) are blunted and reflect decreased 5HT neurotransmission whereas we suggest that CFS may be associated with increased 5HT function.

Serotonin mediated activation of hypothalamic-pituitary-adrenal (HPA) axis in CFS:

Many of the clinical manifestations of chronic fatigue syndrome - debilitating fatigue, an abrupt onset precipitated by a stressor, arthralgia, myalgia, exacerbation of allergic responses, mood and sleep disturbances - are reminiscent of mild glucocorticoid deficiency (Baxter and Tyrell 1981). Patients with hypothyroidism (Kamilaris et al 1987) and seasonal affective disorder (Joseph et al 1991) who present with fatigue, lethargy and atypical depression, show evidence of impaired activation of hypothalamic CRH. Bruno et al (1995) has reported impaired activation of HPA axis to a fasting stressor in patients with post-polio fatigue, a condition that bears striking similarities to CFS. In their study they measured plasma concentration of cortisol and ACTH following a mild stressor effect (fasting) which is known to stimulate the HPA axis (Dallman et al 1993) and found that there was no ACTH elevation in subjects reporting severe fatigue. They suggested that hyposcretion of ACTH may be secondary to decreased production of the hypothalamic secretagogue CRH and vasopressin. Thus post-polio fatigue may be attributable to a polio virus lesion not only in the brain activating system but also in the paraventricular nucleus which reduces the secretion of neuromodulators that stimulate this system (HPA axis). Similarly in patients with CFS, Demitrack et al (1991) conducted a comprehensive dynamic assessment of the hypothalamic-pituitary-adrenal axis and suggested mild central adrenal insufficiency secondary to either a deficiency of CRH or some other secretagogue.

The release of CRH from the paraventricular nucleus is under the control of several classic neurotransmitters, including serotonin (Dinan 1996). These neurotransmitters may influence the receptor content of steroid sensitive cells, and this may constitute an important mechanism by which neurotransmitters modulate steroid dependent processes (Nock et al 1981 Angelucci et al 1981). For example 5HT plays an important role in the negative feedback by glucocorticoids on central HPA-axis function: (a) depletion of 5HT in hippocampal structures may attenuate the negative feedback effects on the axis through downregulation of glucocorticoid (GR) or mineralocorticoid (MR) receptors (Brady et al 1992; Seckl and Fink 1991); and (b) depletion of 5HT causes an enhancement of the negative feedback effects of glucocorticoids on hypothalamic CRH (Feldman and Weidenfeld 1992).

The serotonergic receptor system is complex and evidence is now available to link ACTH release with stimulation of 5HT_{1A} receptor (Gilbert et al 1988). Ipsapirone is a selective 5HT_{1A} agonist and has been shown to produce a dose dependent increase in ACTH and cortisol which is blocked by 5HT_{1A}^{ant} agonists (Lesch et al 1990 and Koenig et al 1988). That 5HT might be involved in the pathophysiology of CFS, as suggested in the first study, it was therefore decided to examine 5HT activation of the hypothalamic-pituitary axis in this syndrome. The study is based on the hypothesis that the abnormalities in hypothalamic-pituitary-adrenal axis function arise from disturbance in serotonergic inputs.

Blunted release of ACTH in response to ipsapirone challenge was demonstrated. This blunting is similar to that previously reported when CRF was administered to subjects with chronic fatigue syndrome (Demitrack et al 1991). The abnormality may be attributed to a number of possible factors: 1. decreased responsivity of 5HT_{1A} receptors at a hypothalamic level. 2. decreased responsivity of CRH receptors on the anterior

pituitary corticotrophs. 3. underactivity of anterior pituitary corticotrophs leading to decreased production and release of ACTH. In order to resolve the site of abnormality, simultaneous measurement of cerebrospinal fluid CRF is required. In case of decreased responsivity of 5HT1A receptors at a hypothalamic level CRF levels would be low compared with healthy controls. A deficiency of CRH could also theoretically contribute to lethargy and fatigue that are cardinal symptoms of CFS. In case of the second and third possibilities levels of CRF are increased. It is unlikely that abnormalities in cortisol feedback either at a pituitary or hypothalamic level are responsible as baseline cortisol levels in the current study were normal. To rule out such a possibility definitely would require monitoring of 24 hours cortisol production in such subjects.

Demitrack et al (1991) reported elevated ACTH levels whilst we were unable to find differences between patients and comparison subjects in terms of baseline ACTH. A detailed 24 hour study of ACTH release is required to throw further light on these differences. Similar blunting of ipsapirone-mediated ACTH release was reported by Lesch et al (1990 b) in a patient with unipolar depression but such blunting was found in the presence of high cortisol levels and they postulated that cortisol might have produced a decreased sensitivity of the postsynaptic 5HT1A receptor. In our study patients were excluded if they had depressive symptoms and we found that cortisol levels did not differ from those found in control subjects.

The view that chronic fatigue syndrome is a masked form of depression therefore, is not supported by our findings. In the first study we examined buspirone induced prolactin release, assuming this to be a measure of 5HT1A receptor responsivity and found an augmented prolactin release in comparison to healthy controls. Similar augmentation in such patients has been reported by Bakheit et al (1992) and in other studies when d-

fenfluramine was used to stimulate prolactin release (Cleare et al 1995). This might indicate that 5HT1A responses show anatomic differences with some responses enhanced and other attenuated in CFS. It is however possible that the differences may be due to differing drug selectivity. If 5HT1A receptors show different anatomic responses such differences could be demonstrated using d-fenfluramine to release both prolactin and ACTH. If differences exist one would expect enhanced prolactin and decreased ACTH release in chronic fatigue syndrome.

The view that the syndrome is simply a variant of affective disorder is not borne out by this study. First, all patients underwent a full psychiatric assessment and none had evidence of significant depressive symptoms or had a past history of major depression. Secondly, patients with major depressive illness show elevations in cortisol levels (Dinan 1994; Checkley 1992), whilst patients here had serum cortisol levels which did not differ significantly from healthy comparison subjects. In fact when 24 hour urinary free cortisol is monitored, cortisol output is seen to be reduced in chronic fatigue syndrome (Demitrack et al 1991). Adrenalectomy in rats increased 5HT1A receptor binding in both the hippocampus and raphe neurones (DeKloet et al 1986). Furthermore the 5HT1 receptor is G-protein linked and the G-proteins are known to be significantly influenced by glucocorticoids (Saito et al 1989). It is therefore possible that the alterations in 5HT1A receptors are partly mediated through differences in cortisol output.

In summary, in patients with CFS, we found no differences in baseline ACTH or cortisol levels but significant attenuation of 5HT1A mediated ACTH responses, using ipsapirone as challenged agent.

Dexamethasone induced growth hormone release: evidence for cerebral steroid receptor resistance:

In chronic fatigue syndrome impaired activation of HPA-axis has recently been reported (Demitrack et al 1991). How chronic fatigue might result from such abnormalities is unknown but it is noteworthy that in patients with panhypopituitarism in whom all hormonal replacements have been made, they still suffer from apathy, lack of drive and chronic fatigue. The basis of this fatigue is unknown and has been suggested to be due to either a deficiency of growth hormone or prolactin (Martin and Reichlin 1987). Some of the features of chronic fatigue syndrome are similar to those seen in adult growth hormone deficiency (Lamberts et al 1992). We therefore studied functional integrity of hypothalamic-pituitary-somatotroph axis and its interaction with HPA-axis as physiological level of corticosteroids is a prerequisite for the normal functioning of hypothalamic-pituitary growth hormone axis (Giustina et al 1989).

Growth hormone is secreted by specific anterior pituitary cells, the somatotrophs, which are influenced by a number of factors. Growth hormone (GH) secretion is stimulated by growth hormone releasing hormone (GHRH) and is inhibited by somatostatin. Negative feedback control is brought about at the pituitary level by somatomedin C, while somatomedin may also act at a high level that is at the hypothalamus to stimulate the secretion of somatostatin. A number of diverse factors act on the release of growth hormone releasing hormone and on somatostatin. Interestingly, exercise and stress, together with β -adrenergic stimuli reduce growth hormone secretion by increasing somatostatin tone while acetylcholine/dopaminergic stimuli and α -adrenergic stimuli increased growth hormone release. The exact role of glucocorticoids in the regulation of growth hormone secretion is unclear. Contrasting with the generally accepted idea that glucocorticoids inhibit growth hormone secretion in man, Casanueva & colleagues

(1990) have been able to demonstrate that dexamethasone alone administered acutely can result in a stimulation of growth hormone release, presumably through alteration in somatostatinergic tone at the level of hypothalamus (Nakagawa et al 1987; Wehrenberg et al 1990). This study is designed hypothesising that abnormalities in hypothalamic pituitary-GH axis stem from impaired activation of HPA axis. We made use of the fact that dexamethasone, the synthetic glucocorticoid when administered acutely stimulates type 2 cerebral steroid receptor and promotes growth hormone release (Casanueva et al 1990).

In this study abnormalities in growth hormone level have been found between healthy controls and patients with both chronic fatigue syndrome and depression. Baseline growth hormone levels in patients with chronic fatigue syndrome and depression were not significantly different but consistent with a trend towards lower level of GH. This findings is in agreement with that of Bennett et al (1992) who described lower levels of growth hormone mediator-somatostatin C (IGF-1) taken at random in 70 women with fibromyalgia/CFS compared with controls.

Another condition which bears a striking similarity to CFS, is the post-polio syndrome. In this condition, in which there is new weakness and incapacitating fatigue developing in patients who have had previous polio, low levels of somatomedin C and GH have been found (Gupta et al 1992). Growth hormone levels decline following puberty although the spontaneous growth hormone secretion rate does not differ significantly over the age range 20-40 years (Finkelstein et al 1972). Whilst the mean age of healthy controls was a little below that of the other two groups, we do not feel that this is important or that the differences found are a result of age. Furthermore in Bennett's study (1992) the patient and control groups were well matched for age and the differences in somatomedin C were still observed. It is not apparent whether these

changes are components of a primary pathological process or are acquired secondary to some of the behavioural aspects of CFS and depression such as reduced physical activity. We did not collect data on current physical activity levels in this study, although reduced physical activity is a criteria for CFS. It is important to extend this work to include control subjects who are more closely age matched to the patients and to ascertain levels of activity in all subjects.

We also observed abnormal responses in growth hormone production to administered steroids in both patients with CFS and depression. Acute administration of dexamethasone (DEX), a synthetic glucocorticoid, by acting on glucocorticoid receptors (GR) in the brain, reduces somatostatin tone and increases serum levels of growth hormone. How steroids are administered, e.g. acutely or chronically, will have an effect on how steroid receptors on certain neurones in the cortex, particularly the limbic system, and the periventricular nucleus of hypothalamus, react. There is receptor plasticity, i.e. changes can occur in neuronal anatomy, physiology or biochemistry of such receptors in response to a variety of stimuli and this plasticity leads to a different response to acute or chronically administered steroids (Casanueva et al 1988).

In this study, the effects of acute administration of dexamethasone on GH release are shown to be markedly reduced in patients with CFS and depression. This abnormality is compatible with a decreased responsivity of cerebral glucocorticoid receptors and fits well with the hypothesis that CFS may be a form of Addison's disease of the brain (Demitrack et al 1991). The high baseline cortisol levels seen in depression may explain the blunted responses by increasing somatostatic tone, (Wehrenburg et al 1990, Lima et al 1993) whereas in patients with CFS cortisol levels did not differ significantly from the healthy comparison group (as measured in the previous study) and low levels have been reported by Demitrack and co-workers (1991) in their studies. It can therefore be

hypothesised that decreased responsivity of steroid receptors is independent of the serum cortisol level (disturbance of HPA system) and receptors have lost their plasticity and become resistant.

In order to confirm this, in the second phase of the study metyrapone, inhibitor of 11 β -hydroxylation was administered prior to dexamethasone challenge in order to block glucocorticoid synthesis and upregulate brain steroid receptors (Chao and McEwan 1989). Despite reduction in cortisol levels in patients with CFS and depression after metyrapone administration, there was no further rise in the production of GH compared with controls. These findings confirm the assumption of cerebral steroid receptor resistance in CFS as does the single case reported by Bronnegard et al (1986), who presented with intermittent fatigue and a tendency for hypotension and was found to have primary cortisol resistance associated with a thermolabile glucocorticoid receptor. Similarly mutations within the glucocorticoid receptor (GR) gene can cause the clinical syndrome of familial glucocorticoid resistance (Hurley et al 1991). Although we cannot explain the mechanisms involved in the pattern of glucocorticoid sensitivity observed in patients with CFS and depression, the genetic influences that determine the sensitivity to glucocorticoid (Becker et al 1976) appear to be superseded by disturbances of the HPA system.

Desipramine induced GH release:

To date little is known about the central noradrenergic functioning in this syndrome. However in a comprehensive study of monoamine metabolism in CFS, Demitrack et al (1992) found lower levels of plasma norepinephrine metabolite - 3 methoxy - 4 hydroxyphenethyleneglycol (MHPG) in CFS patients compared with controls. Plasma free MHPG was used as an index of central noradrenergic (NE) turnover because it has

been shown to reflect brain MHPG, the major metabolite of brain NE (Maas et al 1979). Although the proportion derived from central noradrenergic activity is controversial, with estimates ranging from 20% (Blombery et al 1980) to 60% (Maas et al 1979). On this assumption it can be hypothesised that central noradrenergic function is impaired in patients with CFS. The most widely studied test of central noradrenergic α_2 receptor functioning in man is the clonidine/GH challenge test. α_2 adrenergic receptor is found both presynaptically and postsynaptically on noradrenergic neurones in a variety of brain regions (U'Prichard et al 1979; Morris et al 1981). Growth hormone release in response to clonidine is mediated by postsynaptic α_2 adrenergic receptors (Terry and Martin 1981). Dinan and Barry (1990), in their study in depressives used desipramine, a monoamine reuptake inhibitor, to assess the function of postsynaptic α_2 adrenergic receptors. Desipramine by blocking the reuptake of noradrenaline and indirectly stimulating α_2 adrenergic receptors brings about growth hormone release (Laakmann et al 1986).

We used the same neuroendocrine challenge test to assess the central noradrenergic function in patients with CFS. Blunted release of growth hormone in response to desipramine challenge was demonstrated in a cohort of patients with chronic fatigue syndrome. The abnormality may be attributed to a number of possible factors: 1. presynaptic noradrenaline (NE) hypersecretion resulting in postsynaptic α_2 adrenoceptor downregulation. 2. reduced sensitivity of somatotroph to GHRH stimulation as desipramine induced GH secretion involves intermediate stimulation of the growth hormone releasing hormone (GHRH). 3. somatomedin C resulting in negative feedback on the production of GH. It is unlikely that the sensitivity of somatotroph is affected as in two preliminary studies (unpublished data), in patients with CFS, administration of GHRH produced an increase in GH release. On the other hand, somatomedin-mediated feedback inhibition is not possible in the presence of the well

documented lower level of somatomedin C in this syndrome (Bennett et al 1992). Moreover, in order to exclude interference by fluctuations of ovarian hormones during menstrual cycle, all patients and controls were tested during the early follicular phase when ovarian secretion is minimal. Finally, we concluded that blunted growth hormone response to desipramine is due to α_2 postsynaptic adrenoceptor downregulation secondary to a hypernoradrenergic state.

This result is consistent with the previous reports of lower levels of plasma 3 methoxy 4 hydroxyphenethyleneglycol (MHPG) in patients with CFS (Demitrack et al 1992) suggesting a decrease in NE turnover. Norepinephrine turnover is determined by NE neuronal impulse flow and the amount of NE released and metabolised per nerve impulse (McMillen et al 1980). Due to increased synaptic NE (hyper-noradrenergic state) neuronal impulse flow is reduced and hence NE turnover is decreased. These results are in keeping with the previous reports of blunted growth hormone responses in depressed patients (Checkley et al 1981; Charney et al 1982; Checkley et al 1984; Dinan and Barry 1990). Similarly, postsynaptic α_2 adrenoceptors subsensitivity, as revealed by the blunted growth hormone response to clonidine stimulation is reported in patients with panic disorder (Charney and Heninger 1986; Curtis et al 1989; Nutt et al 1989).

In summary, growth hormone responses to desipramine are significantly attenuated in patients with CFS indicating α_2 - postsynaptic adrenoceptor downregulation secondary to hyperadrenergic state and a decrease in central noradrenergic function. These findings are not specific to CFS as in patients with depression (Dinan and Barry 1990) and panic disorder (Curtis et al 1989; Nutt et al 1989) they are similarly blunted. This test therefore cannot be used by itself as a specific diagnostic test for CFS.

Pyridostigmine Induced Growth Hormone Release:

CFS is relatively common in farmers and agricultural workers exposed to sheep dips and insecticides containing organophosphates (Behan and Haniffah 1994). Psychiatric sequelae has also been reported due to chronic exposure to these insecticides (Gershon and Shaw 1961). Pharmacologically organophosphorus insecticides are cholinesterase inhibitors which prolong and intensify the effects of acetylcholine. There is convincing evidence for the role of acetylcholine in the regulation of mood, psychomotor activity and sleep (Schuberth 1978; Janowsky and Davis 1979). It has also been reported that centrally acting cholinergic drugs produce sleep changes characteristic of major depression (Sitaram et al 1977).

We therefore hypothesised that sleep disturbances and neurobehavioural symptoms in patients with CFS might be due to central acetylcholine neurotransmitter function abnormalities. We tested this hypothesis using a neuroendocrine challenge paradigm. The same approach has been used in two previous studies to assess central ACh neurotransmitter function. Risch (1982) found that physostigmine caused significantly greater increases in β -endorphin in depressed as compared to normal subjects. In the second study O'Keane et al (1992) reported augmented GH release in response to another cholinesterase inhibitor pyridostigmine in depressive illness. We used the same pharmacological probe as it is well tolerated and its mechanism of action in the release of growth hormone is well understood. ACh promotes GH secretion by activating central muscarinic receptors, rather than nicotinic receptors as stimulation of nicotinic receptors by nicotine failed to alter the GH releasing ability of the potent cholinergic agonist eserine (Casanueva 1983). Along with its central action, it may also act at the level of the median eminence or the pituitary, as shown by the finding that methscopolamine, a cholinergic antagonist incapable of crossing the blood brain barrier,

inhibits sleep and insulin-related growth hormone release (Mendleson et al 1978). The inhibition of GRH-induced GH release in man by the muscarinic cholinergic antagonist pirenzepine could also be explained by a pituitary site of action.

Pyridostigmine, by enhancing ACh neurotransmission, causes increased secretion of GH from the somatotrophs by reducing somatostatin release from the somatostatin containing neurones in the periventricular nucleus of hypothalamus (Ross et al 1987). In this study we found an augmented growth hormone response to pyridostigmine in patients with CFS compared to healthy controls. Pyridostigmine is a cholinesterase inhibitor and thereby prolongs and intensifies the effects of acetylcholine, an important neuromodulator of growth hormone release. Growth hormone secretion is primarily regulated by two hypothalamic peptides, GH releasing hormone (GHRH) and somatostatin with stimulating an inhibitory influence on the anterior pituitary somatotrophs respectively, with a negative feedback effect exerted by GH on its own secretion. Ross et al (1987) suggested that this negative feedback effect is mediated through hypothalamic somatostatin release and this somatostatin secretion is under inhibitory cholinergic control. This is confirmed by studies in rats demonstrating a complete inhibition of GH responses to cholinergic drugs if hypothalamic somatostatin is selectively depleted (Locatelli et al 1986).

This increased growth hormone secretion seen in a group of patients with CFS may be attributed to a number of factors. 1. Increased sensitivity of somatotrophs to GHRH stimulation as pyridostigmine induced growth hormone secretion involves intermediate stimulation of GHRH. 2. Hyperresponsivity of cholinergic receptors as hypothalamic level, resulting in greater decrease in somatostatin tone and a bigger surge in GH release from the anterior pituitary. It is unlikely that the sensitivity of somatotroph is affected in CFS as in two preliminary studies (unpublished data) administration of GHRH produced

normal increase in GH release. Acetylcholine hyperresponsivity at hypothalamic level is the most likely explanation for the increased growth hormone secretion and could be caused by presynaptic autoreceptor sub sensitivity; upregulation or hyperresponsivity of post-synaptic receptor or abnormalities in intracellular second messenger systems coupled to the ACh receptor. This finding of ACh hyperresponsivity in patients with CFS may be partly responsible for sleep disturbances and neurobehavioural symptoms of this syndrome and is in keeping with the clinical observation that anticholinesterase administration (Bowers et al 1964) and physostigmine, a cholinomimetic, produce behavioural changes and anergic symptoms, very much similar to that of CFS.

As stated in the methods section we tested all premenopausal women in the early follicular phase of menstrual cycle as growth hormone responses to pyridostigmine are profoundly influenced by the menstrual cycle phase due to changes in sex steroid hormones (O'Keane and Dinan 1992).

In summary, growth hormone responses to pyridostigmine are significantly enhanced in patients with CFS and reflect increased sensitivity of ACh neurotransmitter function. These findings are not specific to CFS as increased cholinergic sensitivity has also been reported in depression (O'Keane et al 1992). This cholinergic overdrive in CFS is probably due to monoamine (NE) depletion which as reported in the previous study is consistent with the cholinergic-adrenergic balance hypothesis of depression (Janowsky et al 1972) it may be the reason why a few of the symptoms of both disorders overlap.

CFS is also known as post-viral fatigue syndrome. Over the years several viruses have been postulated to be of aetiological significance, including EBV and enteroviruses such as the coxsackie B group. Other viruses implicated include herpes simplex, human herpes virus type 6, varicella zoster and rubella (for detail see under section aetiology).

None of these viruses has been definitively linked to this disorder, although viral genomic material has been found in the brain of a fatal case (McGarry et al 1994), suggesting that selective cerebral involvement could contribute to altered neurological and neuroendocrine function. Animals infected with viruses, for instance, neonatal mice with neuronal infection by lymphocytic choriomeningitis virus (LCMV) show decreased production of neurotransmitters. In neuroblastoma cells chronically infected with LCMV, there is no cytopathic effect, cloning efficiency is normal, but there is however, a complete cessation of the normal production of acetylcholine (Oldstone et al 1991). This hypothesis of persistent viruses causing altered cellular function, without cytopathic effect has been proposed as a possible mechanism to explain chronic fatigue syndrome (De La Torre et al 1991). Therefore it can be postulated that the hypersensitivity of ACh receptors in patients with CFS is due to decreased production of ACh neurotransmitter as a result of viral persistence.

Growth hormone release in response to dopamine agonist, bromocriptine.

There is considerable evidence to suggest that brain monoamines including noradrenaline (NE), dopamine (DA) and serotonin (5-HT) are significant determinants of mood and behaviour (Bunny et al 1971). Behavioural alterations in patients with basal ganglia lesion has also been reported (Bowen 1976). Bruno et al (1995) described the role for the basal ganglia in the generation of fatigue in patients with post-polio syndrome, a condition that bears striking similarities to CFS. In the first study we also postulated dopaminergic involvement in enhanced prolactin release to buspirone (5-HT_{1A} agonist & dopamine antagonist) in patients with chronic fatigue syndrome. To test this hypothesis further we studied the dopamine agonist, bromocriptine induced growth hormone release in patients with chronic fatigue syndrome.

There is considerable evidence that dopamine is an important modulator of growth hormone release in man (Camanni et al 1977). Apomorphine and bromocriptine and potent GH secretogue (Wass 1983), and both enhance GHRH induced GH release (Delitala et al 1987), probably by inhibiting the release of endogenous somatostatin from the median eminence (Vance et al 1987). Others have suggested an inhibitory effect of DA on growth hormone release (Arce et al 1991) probably by decreasing noradrenergic mediated GH (Kuchel et al 1987). Therefore exact mechanism (s) of action for dopaminergic agents in stimulating the release of growth hormone in man are not entirely clear and requires further investigations.

We studied the post-synaptic DA receptor by means of a neuroendocrine strategy by quantitatively examining a DA mediated post-synaptic function. We have done this by determining the GH responses to a standardised dose of the DA agonist bromocriptine which acts both centrally and peripherally. We found no differences between patients with CFS and healthy controls. The results, of normal GH release, are in keeping with the previous reports of apomorphine induced GH release in depressed patients (Caspar 1977; Jimmerson and Post 1984), whereas enhanced responses were reported in acute schizophrenia (Hirschowitz et al 1982, 1986). On the basis of this, we concluded that buspirone-induced enhanced prolactin release as previously reported is mediated through serotonergic transmission rather than dopamine mediated.

Bromocriptine produced no severe side effects and was in general very well tolerated. Four subjects reported slight dizziness, headache and nausea.

To summarise, we found that plasma GH responses to bromocriptine challenge were normal and hence DA neurotransmitter function in patients with CFS is unaffected.

Baclofen Induced Growth Hormone Release:

Gamma-aminobutyric acid (GABA) is one of the major inhibitory neurotransmitter in the human brain (Sieghart 1989). The inhibitory action is mediated by GABA receptors, activation of GABA (A) receptor induces the fast inhibitory post-synaptic potential (IPSP) and GABA (B) slow inhibitory post-synaptic potential (IPSP) because of different underlying molecular mechanisms.

Involvement of GABA neurons in the control of affect and emotion has recently been proposed. Many studies have implicated GABA in the pathophysiology of depressive illness (Sanger et al 1986) and trials have shown that the selective GABA agonist progabide and fengabine display a therapeutic action in depressive disorders (Musch 1986). The view that CFS is a form of depressive disorder, the sensitivity of GABA (B) receptors was assessed via the growth hormone response to baclofen to find out the role, if any, of GABAergic mechanisms in the pathophysiology of CFS.

Hypothalamic GABA (B) receptors have been shown to be involved in the modulation of human GH secretion (Koulu et al 1979; Pasariello et al 1982; Monteleone 1988). The exact mechanism by which this occurs is still unknown. Murakami et al (1985) have observed that passive immunisation with anti-GHRH antibodies abolishes the stimulatory influence of GABA on the somatotroph axis, implying that GHRH is essential for the GABA induced release of GH. Others have claimed that GABA's GH releasing capabilities are due to its ability to inhibit somatostatin release (Steardo et al 1986; Twery & Gallagher 1990). On the basis of these observations it is concluded that GABA induced GH release may be a consequence of increased GHRH or reduced

somatostatin (SS) activity. Baclofen, a direct GABA (B) agonist crosses the blood brain barrier (Naik et al 1976) and acts centrally to increase GH release.

In the present study, plasma GH response to baclofen was evaluated in a sample of healthy volunteers and CFS patients. Acute baclofen administration induced a clear cut increase in plasma GH in both healthy subjects and patients with CFS in agreement with previous reports by different groups (Koulu et al 1979; Monteleone et al 1988). There was a difference in the GH response to baclofen between males and females, the latter had a greater response due to augmented effect of oestradiol on growth hormone release (Lucey et al 1991).

No difference was found between healthy subjects and CFS patients with regard to GH response to acute GABAergic challenge. These findings suggest that GABAergic mechanisms are not involved in the pathophysiology of CFS. Similarly, in depression Monteleone et al (1990) found no difference between patients and control subjects. Other investigators (O'Flynn and Dinan 1993) have reported blunted responses in depressed patients on the basis of high cortisol levels as chronic steroid exposure inhibit growth hormone release by enhancing somatostatin tone.

The view that chronic fatigue syndrome is a form of depressive disorder is not supported by the findings of this study. In depression GH responses to baclofen were blunted indicating lower than normal responsivity of type B- γ -aminobutyric acid receptors whereas we suggest non-involvement of GABAergic mechanisms in CFS.

CONCLUSION:

In the first study we examined serotonin function in CFS using 5-HT_{1A} receptor agonist buspirone. Prolactin responses to this agent were augmented in a group of patients with

CFS compared to healthy controls. In the second study we looked at the interaction between serotonergic neurotransmission and the function of the HPA axis. We found no difference in baseline ACTH and cortisol levels in CFS but a significant attenuation of ACTH release in response to the 5-HT₁ agonist, ipsapirone.

In the third study, GH responses to dexamethasone were examined in two phases, before and after metyrapone administration. Blunted responses were recorded in each phase, considered to be due to cerebral steroid receptor resistance. Growth hormone responses to the anticholinesterase pyridostigmine and the monoamine reuptake inhibitor desipramine were examined and found to produce augmented and blunted responses respectively in patients with CFS compared with healthy subjects. Finally, growth hormone responses to the dopamine agonist bromocriptine and the GABA (B) agonist baclofen were measured and found to be normal.

In summary, in CFS, 5-HT receptors are upregulated and 5-HT function is increased. Cerebral steroid receptors are resistant and activation of HPA-axis to serotonergic input is impaired. α ₂-post-synaptic adrenoceptors show decreased sensitivity with a decrease in central noradrenergic function while cholinergic receptors are hypersensitive. Dopamine (DA) and GABA neurotransmitter systems are not involved in the pathophysiology of chronic fatigue syndrome. These neuroendocrine abnormalities may help to explain some of the pathophysiological features of CFS.

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