THE EFFECT OF DRUGS AND EXERCISE ON INTERMEDIARY METABOLISM AND ON THE RELEASE OF GROWTH HORMONE AND PROLACTIN IN MAN

by

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being a thesis submitted for the degree of Doctor of Philosophy in the University of Glasgow (November, 1977)

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ACKNOWLEDGEMENTS

I am grateful to Professor R. M. S. Smellie, Catchart Professor of Biochemistry in the University of Glasgow, for encouragement with this work. I would also like to thank Professor J. A. Simpson, Professor of Neurology, in whose department most of the work was carried out.

Dr. S. R. Bloom, Royal Postgraduate Medical School, London, provided results of analysis of pancreatic glucagon. Mrs. I. McKenzie and Miss. J. Gray performed the analysis of serum DPH levels and also provided some technical assistance. I would like to thank the Department of Medical Illustration for providing photographs of figures and Mrs. M. McColl who typed the thesis.

Dr. E. H. Bennie, consultant psychiatrist, Levendale Hospital, Glasgow, encouraged much of the work in this thesis and arranged for a number of his patients to take part in investigations. I would like to thank Professor R. H. Johnson, Dean and Professor of Medicine, University of Otago, New Zealand for all the encouragement and guidance he has shown in the preparation of this thesis. The work described in this thesis was made possible by a grant to Professor Johnson from the Secretary of State for Scotland.

DECLARATION

The various studies presented in this thesis are all original studies carried out by myself. The investigation described in chapter 4 has already been presented in an M.Sc. thesis (University of Glasgow, 1976) and is included in the present thesis because it contributes to the argument that hypothalamic/pituitary function may be impaired in alcoholics.

SUMMARY

All the studies presented in this thesis involve aspects of the regulation of hGH and hPRL release. The first two sections describe studies of the effects of diphenylhydantoin (DPH) and alcohol and the third section describes studies of hypothalamic regulation of hGH and hPRL release in normal subjects and in patients with Huntington's chorea.

Studies of the effects of DPH demonstrate that this drug potentiates the hGH response to exercise (Chapter 1). Glucose loading demonstrated impaired glucose tolerance and insulin release in epileptics receiving long term treatment with DPH (Chapter 2). The effect of alcohol on hypothalamic/pituitary function was investigated by examining the effect of alcohol on hGH and hPRL release (Chapters 3, 4). Alcohol increased the hGH response to exercise in normal subjects suggesting that alcohol alters hypothalamic/pituitary regulation of hGH release. The hGH responses to exercise and insulin induced hypoglycaemia were impaired in a number of alcoholics and clinical evidence suggested that the presence of an impaired hGH response may be related to alcohol withdrawal (Chapters 5 and 6).

The investigations described in Section III provide evidence that hGH and hPRL release is influenced by hypothalamic dopaminergic, noradrenergic and serotonergic receptors. The dopamine agonist bromocriptine produced significant elevation of hGH concentrations in normal subjects and suppressed both hGH and hPRL levels in a patient with acromegaly (Chapter 7). Studies of the effects of the phenothiazine derivative, fluphenazine, demonstrate that this drug elevates basal hPRL concentrations, enhances the hPRL response to insulin induced hypoglycaemia and impairs the hGH response to this stimulus (Chapters 8 and 9). These effects of fluphenazine on hGH and hPRL release are consistent with the dopamine receptor blocking properties of phenothiazine drugs. The intravenous administration of the noradrenaline receptor agonist clonidine was associated with elevation of hGH and suppression of hPRL concentrations (Chapter 10). Administration of 5-hydroxy-L-tryptophan, the precursor of serotonin, produced a significant rise in both hGH and hPRL concentrations (Chapter 11).

The regulation of release of hGH and hPRL by hypothalamic peptide factors was studied by investigating the effect of

somatostatin on hormonal and metabolic changes during and after exercise in normal subjects (Chapter 12). The growth hormone release inhibiting property of this peptide was confirmed and the suppression of the hGH response did not appear to have any marked effect on fat mobilisation during exercise.

The presence of an earlier hGH response to insulin induced hypoglycaemia was confirmed in patients with Huntington's chorea (Chapter 13). Further studies of the hGH and hPRL responses to bromocriptine, clonidine and 5-hydroxy-L-tryptophan in patients with this disorder demonstrated significantly reduced hGH responses to these three agents and a greater hPRL response to 5-HTP. The results do not provide evidence of increased sensitivity of hypothalamic catecholaminergic or serotonergic receptors stimulating hGH release in patients with Huntington's chorea.

ABBREVIATIONS

ACTH	adrenocorticotrphic hormone
ADP	adenosine diphosphate
ATP	adenosine triphosphate
FFA	free fatty acids
HBDH	hydroxybutyrate dehydrogenase
hGH	human growth hormone
hPRL	human prolactin
5-HTP	5-hydroxy-L-tryptophan
LDH	lactate dehydrogenase
min	minutes
NAD(H)	nicotinamide adenine dinucleotide (reduced)
PEP	phosphoenol pyruvate
PK	pyruvate kinase
SEM	standard error of the mean

UNITS

g	gram
Kg	kilogram (10^{3}g)
mg	milligram (10^{-3}g)
ug	microgram (10^{-6}g)
ng	manogram (10^{-9}g)
l	litre
ml	millilitre (10^{-3}l)
µl	microlitre (10^{-6}l)
u	unit
mu	milliunit (10^{-3}u)
uu	microunit (10^{-6}u)
mol	mole
mmol	millimole (10^{-3}mole)
umol	micromole (10^{-6} mole)
Kp	kilopond
m	metre

Conversions

16 watts = 100 kpm

INTRODUCTION

This thesis describes the work carried out by myself while working as a research student in the Department of Neurology of the University of Glasgow. The introduction provides the background to the various studies and the results have been organised into three sections; (I) studies of the effects of diphenylhydantoin, (II) studies of the effects of alcohol and (III) studies of the regulation of release of growth hormone (hGH) and prolactin (hPRL). Within each section the results are presented and discussed separately in each chapter. A final chapter contains a general discussion and suggestions for future research.

All the studies included in this thesis involve aspects of the regulation of hGH and hPRL release. In some investigations intermediary metabolism was also studied particularly in relation to known metabolic stimuli affecting hGH and hPRL release. Growth hormone release was examined in subjects treated with various drugs thought to have an action on the hypothalamic/pituitary axis and regulation of hGH and hPRL release was investigated in normal subjects and patients with evidence of altered hypothalamic/pituitary function.

GENERAL

Animals grow, differentiate, reproduce, respond and maintain themselves by virtue of a great number of individual integrated biochemical reactions that together constitute the metabolism of the animal. It is the integration and regulation of these reactions that is essential for normal function and survival. The concept that internal secretions can regulate internal function in animals dates from the studies of Claude Bernard (1855) on hepatic glycogen. Baylis and Starling (1904) published their work on secretin and demonstrated that a specific chemical messenger or hormone could be secreted by an organ or tissue into the blood stream and effect the function of another organ or tissue. A number of hormones have now been found to regulate metabolism by modifying some aspect of cellular metabolism and the tissues which release these hormones are known collectively as the endocrine system. The nervous system and the endocrine system are both linked developmentally and in some cases the endocrine system acts as an extension of the nervous system. The pituitary gland or hypophysis is of major importance in the endocrine system as it links the central nervous system to the endocrine system and as a number of pituitary hormones regulate release of hormones from other endocrine tissues.

The Adenohypophysis

The hypophysis or pituitary gland develops embryologically from Rathke's pouch, an evagination of epithelial tissue that will form the roof of the mouth and a down growth of neural ectoderm from the tissue that will eventually form the floor of the third ventricle. It is possible to divide the gland histologically into the highly vascularised anterior pituitary or adenohypophysis which receives blood from the portal plexus system (Harris and Green, 1947), and the posterior or neurohypophysis with its rich supply of branching unmyelinated nerve fibres most of which originate in the hypothalamus (Fisher, Ingram and Ranson, 1938).

The sixteenth century anatomist Vesaluis believed that the pituitary gland functioned as a filter or trap whereby the phlegm or pituita, the waste product of the transformation in the cerebral ventricles ofvital spirits into animal spirits, found its way into the nose or pharynx (Singer, 1952). This early interpretation of the relationship between the gland and the brain may be somewhat incredible today but much of the twentieth century research work into the regulation of release of pituitary hormones still involves the concept of a 'flow of secretion' from the brain to the gland. Crowe, Cushing and Hamons (1910) reviewed the early work on hypophysectomy. Most of the early studies were unsatisfactory as the animals usually died immediately afterwards and few observations were made on animals that did survive for any

length of time. Smith (1930) carried out successful hypophysectomy in young rats and noted end organ atrophy and dwarfism. The effect of excising the adenohypophysis is now known to include atrophy of the gonad, thyroid and adrenal cortex, failure of milk synthesis and arrest of growth. Recognition of these effects stimulated the search for at least six anterior pituitary hormones. At least seven distinct anterior pituitary hormones have been identified. Four of these hormones follicle stimulating hormone (FSH), luetinising hormone (LH), thyroid stimulating hormone (TSH) and adrenocorticotrophic hormone (ACTH) act on other endocrine glands. However, the remaining three hormones, growth hormone, prolactin and melanocyte stimulating hormone (MSH) exert their effects directly on target tissues.

The identification of the portal plexus system and its close association to nerve fibres from the hypothalamus led to the suggestion of a neurohormonal junction between the hypothalamus and glandular cells of the adenohypophysis (Harris, 1955). An essential feature of the early ideas of hypothalamic control of the adenohypophysis was that nerve impulses released humoral agents into the portal plexus system which stimulated secretion of anterior pituitary hormones. Evidence for the existence of these humoral factors has come mainly from observations of the effects of lesions in the

hypothalamus and the effects of extracts of hypothalamic tissue on release of hormones from adenohypophysis tissue 'in vitro'. A hierarchy of control has been suggested in which the anterior pituitary hormone promotes secretion of target organ hormone which can feedback on the hypothalamus or pituitary to regulate further release of pituitary hormone. The control of growth hormone and prolactin release was thought to involve feedback of the pituitary hormone itself on the hypothalamus or pituitary (Greer, 1957; Guillemin, 1964). An important feature of these ideas of pituitary hormone control was that the various mechanisms operated independently for each hormone.

Growth hormone

Growth hormone is synthesised by specific anterior pituitary cells distinct from those that synthesise other pituitary hormones including prolactin (Martin, 1973). Growth hormone is a single chain polypeptide containing 190 amino acid residues the sequence of which has now been elucidated (Li, 1972). It accounts for 4-10% of the wet weight of the anterior pituitary in the human adult (5-15 mg/gland) and circulates in the plasma in an unbound form. The half life of hGH has been estimated at 17-45 minutes with metabolic clearance occurring principally by hepatic uptake (Cameron, Burger and Catt, 1969).

Studies in humans have demonstrated that hGH can enhance uptake of FFA and decrease glucose uptake (Rabinowitz, Klassen and Zierler, 1965). It is released in response to a fall in blood glucose (Roth, Glick, Yalow and Berson, 1963; Hunter, Fonseka and Passmore, 1965a), a fall in plasma FFA (Hartog, Havel, Copinschi, Earll and Ritchie, 1967; Irie, Sakuma, Tsushima, Shizume and Nakaro, 1967) and as a result of diurnal variation (Glick and Goldsmith, 1968). The secretion of hGH appears to produce metabolic adjustment in fat and carbohydrate metabolism such that there is decreased glucose use, maintenance of glycogen stores, increased oxidation of fat and maintenance of blood glucose levels in addition to its better known effects on protein metabolism. The mechanism by which growth hormone regulates carbohydrate and fat metabolism is unclear but it seems likely that it interferes with the phosphorylation of glucose and this may involve FFA or ketones which are known to inhibit indirectly the phosphorylating enzyme hexokinase.

Prolactin

Until comparatively recently the existence of a distinct human prolactin hormone had not been conclusively demonstrated although there was considerable clinical evidence to support the existence of this hormone (Forbes, Henneman, Griswold and Albright, 1954). The similarity in physical, chemical and biological properties of growth hormone and prolactin has

contributed to the difficulty in isolating human prolactin. In addition, pooled pituitary extracts contain large concentrations of growth hormone and small amounts of prolactin (Guyda and Friesen, 1971). Recent studies have, however, provided overwhelming evidence to support the existence of prolactin in primate species (Nichol and Nichols, 1971).

The biological role of prolactin in various vertebrate species is only now beginning to be defined. An amazing number of activities have been ascribed to this hormone including mammotrophic, lactogenic, osmoregulatory, diabetogenic and luteotrophic effects (Nicol and Bern, 1972). A role for prolactin in lactogenesis in man is fairly well established (Tyson, Khojandi, Huth and Andreassen, 1976) and intravenous infusion of prolactin produces marked lipolysis in man (Berle, Finsterwalder and Apostolakis, 1974). Exercise and hypoglycaemia are both known to stimulate hPRL release (Noel, Suh, Stone and Frantz, 1972).

Regulation of hGH and hPRL release

There is considerable evidence from a large number of studies in man and animals suggesting that hGH and hPRL release is controlled by the hypothalamus. This evidence includes the observations that pituitary stalk section prevents the normal rise in hGH in response to hypoglycaemia

(Brown and Reichlin, 1972) and that median eminence lesions prevent growth hormone responses in monkeys (Brown, Schalch and Reichlin, 1971). In addition, transection of the pituitary stalk in man (Turkington, Underwood and Van Wyk, 1971) as well as autotransplantation of the pituitary in animals (Everett, 1966) produces elevation of prolactin levels relative to other pituitary hormones. These observations suggest that growth hormone release is under the control of a hypothalamic releasing factor and that the tonic influence of the hypothalamus on prolactin release is inhibitory. However, although there is considerable evidence that peptide hypothalamic hormonal factors regulate hGH and hPRL release none of these factors have been isolated and characterised with the exception of a growth hormone release inhibiting peptide, somatostatin.

In recent years a variety of peptide hormones have been isolated from the mammalian hypothalamus and several, including thyrotrotrophin releasing factor (TRH) luteinizing hormone releasing factor and growth hormone release inhibiting factor have been characterised and synthesised (Marlarkey, 1976). However, recent research has questioned the independence of hypothalamic/pituitary control mechanisms. For example, TRH which stimulates TSH secretion, has also been shown to stimulate hPRL release (Jacobs, Snyder, Wilber,

Utiger and Daughaday, 1971), and hGH release in patients with acromegaly (Saman, Leavens and Jesse, 1974). Somatostatin, a cyclic tetradecapeptide originally isolated from the ovine hypothalamus (Brazeau, Vale, Burgus, Ling, Butcher, Rivier and Guillemin, 1973) can inhibit hGH secretion in response to a number of stimuli (Hall, Besser and Schally, 1973; Hansen, Orskov, Seyer-Hansen and Lundbask, 1973), while also inhibiting secretion of thyrotrophin (Siler, Yen, Vale and Guillemin, 1974) glucagon (Gerich, Lorenzi and Schneider, 1974) and insulin (Chideckel, Palmer and Koerker, 1975). Multiple actions have been described for all the hypothalamic factors so far isolated and the concept of a single hypothalamic factor regulating a single anterior pituitary hormone is no longer tenable.

Regulation of hGH and hPRL release by hypothalamic neurotransmitters

There is growing evidence that hypothalamic neurotransmitters regulate anterior pituitary hormone release. For example, there is evidence for a stimulation of secretion of hGH by the dopamine receptor agonists, apomorphine (Lal, de la Vega, Sourkes and Friesen, 1973; Maany, Frazer and Mendels, 1975), and bromocriptine (Cammani, Massara, Belforte and Molinatti, 1975; Tolis, Pinter and Friesen, 1975) while the drugs chlorpromazine and reserpine

which alter brain catecholamine metabolism impair the hGH response to insulin hypoglycaemia (Sherman, Kim, Benjamin and Kolodny, 1971; Cavagnini and Peracchi, 1971). There is also evidence for serotonergic involvement in hGH release (Imura, Nakai and Yoshimi, 1973). Recent research suggests that there is a hierarchy of hypothalamic control of hGH release. Chlorpromazine and reserpine impair the hGH response to hypoglycaemia but the increase in hGH in response to L-dopa administration is not altered by hyperglycaemia (Boyd, Lebovitz and Pfieffer, 1970). In addition α-adrenergic blockade prevents the hGH response to hypoglycaemia (Blackard and Heidingsfelder, 1968), exercise (Hansen, 1971) and to L-dopa (Kansal, Buse and Talbert, 1972). However, sleep induced hGH release is not altered by adrenergic blockade (Lucke and Glick, 1971). These observations suggest that there is a hierarchy of control of hGH release in the hypothalamus with a final common pathway probably involving the release of growth hormone releasing factor.

There is also evidence that hPRL release is modulated by hypothalamic neurotransmitters. This evidence includes the observations that hPRL levels are decreased by L-dopa (Friesen, Guyda, Wang, Tyson and Barbeau, 1972) and elevated by phenothiazines (Beumont, Gelder and Friesen, 1974; Wiles, Kolakowska, McNeilly, Mandelborte and Gelder, 1976). Studies of specific

dopamine receptor agonists apomorphine and bromocriptine suggest that dopamine inhibits hPRL release (Martin, Lal, Tolis and Friesen, 1974; Del Pozo, Brun, Varga and Friesen, 1972). There is also evidence that serotonergic mechanisms release hPRL (Kato, Nakai, Imura, Chihara and Ohgo, 1973).

There is therefore considerable evidence suggesting that hypothalamic neurotransmitters control hGH and hPRL release. The mechanisms for this action are unknown but it seems likely that modulation of release of hypothalamic peptide release or inhibiting factors is involved. However, as already discussed, only growth hormone release inhibiting hormone has been isolated and characterised, therefore, any discussion of catecholaminergic or serotonergic regulation of the other proposed peptide factors must be speculative. Nevertheless, the effect of hypothalamic neurotransmitters on hypothalamic peptide factors remains an exciting area for future research.

A major objective of the studies described in this thesis was to investigate the effects of various drugs on growth hormone and prolactin release and to determine if there was any evidence for an alteration of hypothalamic/pituitary function. In addition catecholaminergic and serotonergic aspects of hGH and hPRL release were also examined in normal

subjects and in patients with evidence of altered hypothalamic/pituitary function. The following part of this introduction provides the background to the various investigations carried out.

Diphenylhydantoin

Studies in animals have demonstrated that DPH stimulates the pituitary gland and adrenal cortex (Woodbury, 1952). In man DPH administration produces an initial increase in hydroxycorticoid excretion (Costa, Glaser and Bonnycastle, 1955) and it is possible that this effect is due to stimulation of the hypothalamic/pituitary axis. It was therefore decided to investigate the effect of DPH on growth hormone and metabolic changes in response to exercise and glucose loading.

Alcohol and Alcoholism

Oral alcohol ingestion has been reported to elevate hGH (Bellet, Yoshimine, De Castro, Roman, Parmar and Sandberg, 1971) and cortisol (Merry and Marks, 1969; Bellet, Roman, De Castro and Herrera, 1970) in normal subjects and the cortisol response is reported to be absent in patients with pituitary adenomas (Jenkins and Connolly, 1968). This evidence suggests that alcohol has an effect on the hypothalamic/pituitary axis and it was proposed to investigate the effect of alcohol and

exercise on hGH and hPRL release in normal subjects and to study hypothalamic/pituitary function in chronic alcoholics.

Regulation of release of hGH and hPRL

There is evidence that hypothalamic neurotransmitters regulate hGH and hPRL release and some of this evidence has already been discussed. A major objective of this thesis was to investigate catecholaminergic and serotonergic regulation of hGH and hPRL release in normal subjects and to develop techniques for the investigation of hypothalamic neurotransmitter function in patients.

Bromocriptine

Bromocriptine is an active ergot alkaloid with the properties of a dopamine receptor agonist. It was decided to investigate the effect of bromocriptine on hGH and hPRL release in normal subjects and an opportunity also arose to study the responses in a patient with acromegaly.

Phenothiazines

Oral administration of phenothiazines is known to elevate hPRL levels in man (Beumont et al., 1974) and to impair the hGH response to hypoglycaemia (Sherman et al., 1971). Most studies of phenothiazines have involved oral administration of chlorpromazine and it was therefore proposed to investigate

the effects of a depot preparation of fluphenazine on basal hPRL levels and on hGH responses to hypoglycaemia.

Clonidine

Clonidine is an antihypertensive drug which stimulates central noradrenaline receptors. It was decided to investigate the possible importance of noradrenergic mechanisms in the regulation of hGH and hPRL release by examining the response of these hormones to intravenous administration of clonidine.

5-hydroxy-L-tryptophan

Administration of 5-HTP, the precursor of serotonin, is known to elevate brain serotonin levels (Corrodi, Fuxe and Hokflet, 1967). It was proposed to investigate serotonergic regulation of hGH and hPRL release by examining changes in hGH and hPRL levels following intravenous infusion of 5-HTP in normal subjects.

Somatostatin

Somatostatin is a cyclic tetradecapeptide postulated to be a hypothalamic growth hormone release inhibiting hormone (Brazeau et al., 1973). Somatostatin inhibits hGH responses to exercise (Hansen et al., 1973) and also inhibits insulin

(Chidechel et al., 1975) and glucagon release (Gerick et al., 1974). Synthetic somatostatin may be a useful tool in the investigation of the metabolic effects of a number of hormones and it was therefore proposed to study the effect of somatostatin induced suppression of hGH, insulin and glucagon on ketone production and utilisation of metabolic fuels during and after exercise.

Huntington's chorea

Huntington's chorea is an inherited disorder of the nervous system affecting the basal ganglia and in particular the caudate nucleus and putamen (Bruyn, 1968; Earle, 1973). The disorder is characterised by the appearance of involuntary movements or chorea which develop in most cases between the ages of 40-50 years. There is evidence to implicate dopamine in the disorder, as drugs which alter brain dopamine metabolism, for example, haloperidol (Vaisberg and Saunders, 1963) phenothiazines (Candelise, Faglioni and Spinnler, 1973) and tetrabenazine (McLellan, Chalmers and Johnson, 1974) all reduce chorea. Biochemical abnormalities in the brains obtained post mortem from patients who have suffered from Huntington's chorea include a reduction of γ-aminobutyric acid (GABA) concentrations and glutamic acid decarboxylase activity (Perry, Hansen and Kloster, 1973; Bird and Iversen, 1974; Stahl and Svanson, 1974). There is further

evidence for a relationship between GABA and dopamine turnover (Shoulson, Kartzinel and Chase, 1976) and it is possible that the hyperkinetic condition present in the disorder may be related to a change in the relationship between dopamine and GABA. Patients with the disorder show excessive sweating and weight loss and this has been attributed to altered hypothalamic function (Bruyn, 1973). An earlier hGH response to hypoglycaemia has been reported in patients with the disorder and it has been suggested that this earlier response may be related to hypersensitivity of dopamine receptors mediating hGH release (Keogh, Johnson, Nanda and Sulaiman, 1976; Phillipson and Bird, 1976). A major objective of this thesis was to confirm these findings and to investigate catecholaminergic and serotonergic regulation of hGH and hPRL release in patients with the disorder.

METHODS

1. EXERCISE

Subjects exercised on an electric, variable load, Elema Shonander bicycle ergometer (type EM 369). This machine allowed exercise to be performed at a constant known workload. The subjects maintained a relatively steady rate of exercise by monitoring the r.p.m. of the bicycle at intervals. The exercise test performed in this manner was both quantitative and reproducible.

2. ORAL TOLERANCE TESTS AND DRUGS

Glucose tolerance test

A standard oral glucose tolerance test was performed by giving the subject 50g of glucose dissolved in 200 ml of water. Venous blood samples were taken at 30 minute intervals after oral glucose for $2\frac{1}{2}$ hours.

Alcohol administration

To study the effect of alcohol pure ethanol (Burroughs Ltd.) was given orally in a weight related dose (0.5g/kg body weight) and made up to 200 ml with water.

Drugs

The following drugs were used in the various investigations described in this thesis.

Bromocriptine	(Parlodel)	Sandoz Products Ltd.
Clonidine	(Catapres)	Boehringer Ingelheim Ltd
Diphenylhydantoin	(Epanutin)	Parke, Davis & Co. Ltd.
Fluphenazine decanoate	(Modecate)	E. R. Squib & Son Ltd.
5-hydroxy-L-tryptophan	(5-HTP)	B.D.H. Ltd.
Somatostatin (synthetic cyclic somatostatin)		Ayerst Laboratories Ltd.

The method of administration and the dose of drug used is given in the appropriate chapter.

3. BIOCHEMICAL ANALYSIS

Sampling technique

Venous blood samples were withdrawn at appropriate intervals of time from a polythene cannula in an antecubital vein. For each sample taken, 4 ml was added to 5 ml of ice cold 10% $^{w}/v$ perchloric acid in a preweighed tube for the determination of glucose, lactate, pyruvate, acetoacetate, 3-hydroxybutyrate and glycerol. An additional 10 ml of blood was added to a heparinised container for the estimation of plasma free fatty acids, growth hormone, insulin, prolactin and cortisol. Immediately after an investigation samples were weighed, centrifuged at 3,000 r.p.m. for 10 minutes and stored at -20°C until required.

Treatment of denatured blood

The perchloric acid extract was centrifuged (2,500 r.p.m. for 10 minutes), poured into a graduated tube and the volume noted. The extract was neutralised with 20% potassium hyroxide using BDH universal indicator to remove potassium perchlorate precipitate. The volume of the neutralised extract was noted. Biochemical determination of glucose, lactate, pyruvate, 3-hydroxybutyrate, acetoacetate and glycerol were carried out on a known volume of the neutralised extract. A dilution factor was calculated from the volume of blood added to 5 ml of perchloric acid and the change in volume after neutralisation.

4. BIOCHEMICAL METHODS

Blood pyruvate

Pyruvate was estimated by the enzymic method of Hohorst, Kreutz and Bucher (1959). This assay measures the decreased optical density due to the oxidation of reduced nicotinamide adenine dinucleotide (NADH) at 340 nm following the reduction of pyruvate to lactate by lactate dehydrogenase (LDH) at pH 7.0.

$$\text{Pyruvate} + \text{NADH} + \text{H}^{+} \underset{\longleftarrow}{\overset{\text{LDH}}{\longrightarrow}} \text{Lactate} + \text{NAD}$$

Blood lactate

Lactate was estimated by the enzymic method of Hohorst, Kreutz and Bucher, (1959). This assay measures the increase in optical density due to the reduction of NAD at 340 nm following the oxidation of lactate to pyruvate by lactate dehydrogenase at pH 9.5. Pyruvate was removed in the form of its hydrazone.

$$\text{Lactate} + \text{NAD} \underset{\longleftarrow}{\overset{\text{LDH}}{\longrightarrow}} \text{Pyruvate} + \text{NADH} + \text{H}^{=}$$

Blood acetoacetate

Acetoacetate was determined by the enzymic method of Williamson, Mellanby and Krebs, (1962). This assay measures the decrease in optical density due to the oxidation of NADH at 340 nm following the reduction of acetoacetate to 3-hydroxybutyrate by 3-hydroxybutyrate dehydrogenase (HBDH) at pH 7.0.

$$\text{Acetoacetate} + \text{NADH} + \text{H}^{+} \underset{\longleftarrow}{\overset{\text{HBDH}}{\longrightarrow}} \text{3-hydroxybutyrate} + \text{NAD}$$

Blood 3-hydroxybutyrate

3-hydroxybutyrate was determined by the enzymic method of Williamson et al., (1962). This assay measures the increased optical density due to the reduction of NAD at 340 nm following the oxidation of 3-hydroxybutyrate to acetoacetate by (HBDH) at pH 9.5. Acetoacetate is removed in the form of its hydrazone.

$$\text{3-hydroxybutyrate} + \text{NAD} \underset{\longleftarrow}{\xrightarrow{\text{HBDH}}} \text{acetoacetate} + \text{NADH} + H^+$$

Blood glycerol

Glycerol was determined by the enzymic method of Kreutz (1962). Glycerokinase (GK) was used to catalyse the phosphorylation of glycerol to glycerol - 1 - phosphate from adenosine triphosphate (ATP).

$$\text{glycerol} + \text{ATP} \underset{\longleftarrow}{\xrightarrow{\text{GK}}} \text{glycerol-1-phosphate} + \text{ADP}$$

The adenosine diphosphate was rephosphorylated to ATP with phosphoenol pyruvate (PEP) and pyruvate kinase.

$$\text{ADP} + \text{PEP} \rightleftharpoons \text{ATP} + \text{Pyruvate}$$

Pyruvate was then reduced to lactate with LDH and the decrease in optical density due to the coupled oxidation of NADH measured at 340 nm.

$$\text{Pyruvate} + \text{NADH} + H^+ \underset{\longleftarrow}{\xrightarrow{\text{LDH}}} \text{Lactate} + \text{NAD}.$$

The overall reaction is:-

$$\text{glycerol} + \text{PEP} + \text{NAD} + H^+ = \text{glycerol-1-phosphate} + \text{lactate} + \text{NAD}.$$

Plasma free fatty acids

Free fatty acids were estimated in plasma by the colorimetric method of Itaya and Ui (1965) with the modifications of Dalton and Kowalski (1967) for an automated procedure. This method involves the extraction of free fatty acids (FFA) firstly into aqueous buffer then into chloroform. The FFA is estimated in the chloroform extract as the copper soap with diethyldithiocarbamate.

Blood glucose

Blood glucose was estimated by the glucose oxidase method (Bergmeyer and Bernt, 1963) using a Boehringer Biochemical Test Combination (No. 5755) Werner, Rey and Wielinger, 1970. Glucose is oxidised by glucose oxidase to gluconolactone which in aqueous solution is converted to gluconic acid.

$$\text{glucose} + O_2 + H_2O \rightleftharpoons \text{gluconic acid} + H_2O_2$$

In the presence of peroxidase, the hydrogen peroxide oxidises the chromogen with the formation of a dye.

$$H_2O_2 + \text{chromogen} \rightleftharpoons \text{dye} + H_2O$$

The intensity of the dye is proportional to the concentration of glucose and was measured at 440 nm.

Plasma 11-hydroxycorticoids

Determination of 11-hydroxycorticoid concentrations was performed using a fluorimetric method (Mattingly, 1962).

In this method corticosteroids are extracted into dichloromethane and fluorescence of 11-hydroxycorticoids is measured after mixing the dichloromethane extract with fluorescence reagent. In man the principal free-hydroxycorticoid is cortisol although corticosterone is also present in small amounts (Bush and Sandberg, 1953).

Serum Diphenylhydantoin

Serum DPH was measured by gas-liquid chromatography using a Pye Unicam gas chromatograph (Type 104). The method used is a variation of that described by Goudie and Burnett (1973) using p-tolyl phenylhydantoin as an internal standard and on column methylation with trimethyl phenyl ammonium hydroxide.

4. RADIOIMMUNOASSAY

The development of radioimmunoassay techniques has made possible the routine measurement of physiological levels of many peptide hormones in biological fluids. The general principle of these techniques is the competition for a binding site on a fixed amount of anti-hormone antibody between a known amount of radioactively labelled hormone (A*) and an unknown amount of unlabelled hormone (A).

$$A + \text{anti-}A + A^* \rightleftharpoons A : \text{anti-}A : A^* + A + A^*$$

Because of competition, the amount of labelled hormone bound to the antibody will decrease as the concentration of unlabelled hormone increases. The reaction is allowed to go to completion and the antibody-bound hormone is separated from the free hormone and the distribution of radioactivity determined.

Double antibody system

In the double antibody system the antibody hormone complex is separated from the free hormone by a second antibody against the immunoglobulins of the antihormone serum (Morgan and Lazarow, 1963).

Human Growth Hormone

Human growth hormone was measured using a double antibody technique in which the final immunoprecipitate was separated

by filtration (Morgan, 1966; Sorin Gruppo Radiochimica, Italy). The growth hormone concentrations were expressed in terms of the W.H.O. 1st International Reference Preparation of Growth Hormone 66/217 (1 mg = 2 i.u.).

Prolactin

Prolactin was measured using a double antibody technique. The second antibody was fixed on activated cellulose using the Wide method (Wide, 1969; Sorin Gruppo Radiochimica, Italy). (Standard, 1 mg = 40 I.U. MRC 71/22).

Insulin

Insulin was estimated by a single antibody method. The anti-hormone antibody remained in solution but the free hormone is removed by absorption on to charcoal (Hunter and Ganguli, 1971; Yalow and Berson, 1960; Sorin Gruppo Radiochimica, Italy). (Standard, WHO 1st IRP of insulin 66/304).

Glucagon

Plasma samples had 1000 Kallikrein inhibiting units of aprotinin (Trasylol) added for each ml and were frozen (-20^{o}C) for later radioimmunoassay. MRC 69/104 glucagon standard made up in glucagon free plasma was used together with a pancreatic glucagon specific antiserum (Bloom, 1974). This assay gives lower basal glucagon values than those reported by other workers (Vinger and Lefebvre, 1972) because interference from non-specific effects (Weir, Turner and Martin, 1973) has been minimized.

CHAPTER 1

THE EFFECT OF DIPHENYLHYDANTOIN ON THE GROWTH HORMONE AND METABOLIC RESPONSE TO EXERCISE

INTRODUCTION

Diphenylhydantoin (DPH) is known to impair the insulin release in response to glucose loading (Malherbe, Burrill, Levin, Karam and Forsham, 1972) and there is evidence that DPH inhibits insulin release from isolated pancreas (Levin, Booker, Smith and Cradsky, 1970). Such hormonal effects may have important implications for treatment with DPH and it was therefore decided to investigate the effect of the drug on carbohydrate and fat metabolism in response to exercise. In addition, growth hormone release was examined for evidence of any effect of DPH on release of pituitary hormones.

METHODS

Subjects and procedure

Six normal healthy subjects aged 22-40 years (mean 29 years) were investigated on two separate occasions. The subjects were asked to fast overnight before each investigation. On the first occasion the subjects received DPH (500 mg) by mouth overnight and the following morning exercised on a bicycle ergometer at 600 kpm for 30 minutes. On the second occasion the same subjects performed an identical exercise test but received no medication.

Blood samples were taken from a cannula in an antecubital vein before, at 10 minute intervals during exercise and at 5, 15, 30, 60, 90 and 120 minutes after exercise. Blood samples

were analysed for lactate, pyruvate, acetoacetate, 3-hydroxybutyrate, glucose and glycerol. Plasma samples were analysed for FFA and hGH and resting serum samples for DPH. The significance of differences were examined using the Mann-Whitney non-parametric U test for small samples (Mann and Whitney, 1947).

RESULTS

Serum diphenylhydantoin

Serum concentrations of DPH were similar (mean 30.7 μmol/1: range 23.2 - 44.4 μmol/1) and were just below the accepted therapeutic range (40-80 μmol/1) for epileptic patients (Lancet, 1975).

Blood lactate and pyruvate

Basal blood lactate and pyruvate concentrations were similar on both occasions. During exercise blood lactate reached maximum levels after 10 minutes of 3.4 μmol/ml and 3.1 μmol/ml (mean concentrations with and without DPH respectively). Blood lactate concentrations returned to normal resting levels 60 minutes after the end of exercise. The changes in pyruvate concentrations were similar. There was no significant difference between concentrations of lactate or pyruvate at any time during the investigation on the two occasions.

Plasma FFA (Fig 1.I)

Basal plasma FFA concentrations were not significantly different on the two occasions. There was a rise in FFA concentrations towards the end of exercise on the two occasions and mean FFA concentrations after DPH were higher than the corresponding control values after exercise and were significantly greater 15 minutes after exercise ($P < 0.05$).

Blood glycerol (Fig 1.I)

Basal blood glycerol concentrations were similar on both occasions. Exercise was associated with a rise in blood glycerol concentrations and the glycerol concentrations after DPH treatment were significantly greater than the corresponding control values at 5, 15, 30 and 60 minutes after exercise ($P < 0.01$, 0.01, 0.05 and 0.05 respectively).

Total blood ketones (Fig 1.I)

Basal concentrations of total ketones (acetoacetate + 3-hydroxybutyrate) were similar on both occasions. After exercise ketone concentrations rose on both occasions and the concentrations after DPH treatment were significantly greater than the corresponding control concentrations at 15, 30, 60, 90 and 120 minutes after exercise ($P < 0.05$, 0.01, 0.001, and 0.001 respectively).

Blood glucose

Exercise was associated with a similar small fall in glucose concentrations on both occasions but after exercise levels returned quickly towards basal concentrations. There was no significant difference in blood glucose concentrations on the two occasions at any time during the investigation.

Plasma growth hormone (Fig 1.II)

There was no significant difference in basal growth hormone concentrations on the two occasions. Exercise produced a marked

rise in hGH concentrations reaching a peak at the end of exercise on both occasions. Growth hormone concentrations during exercise were significantly greater than the corresponding control concentrations at 10, 20 and 30 minutes ($P < 0.05$, 0.001 and 0.01 respectively).

FIG 1.I Plasma FFA, blood glycerol and total blood ketone (acetoacetate + 3-hydroxybutyrate) concentrations (μmol/ml, mean ± SEM) during and after exercise in six normal subjects with (●——●) and without (■---■) administration of DPH (500 mg).

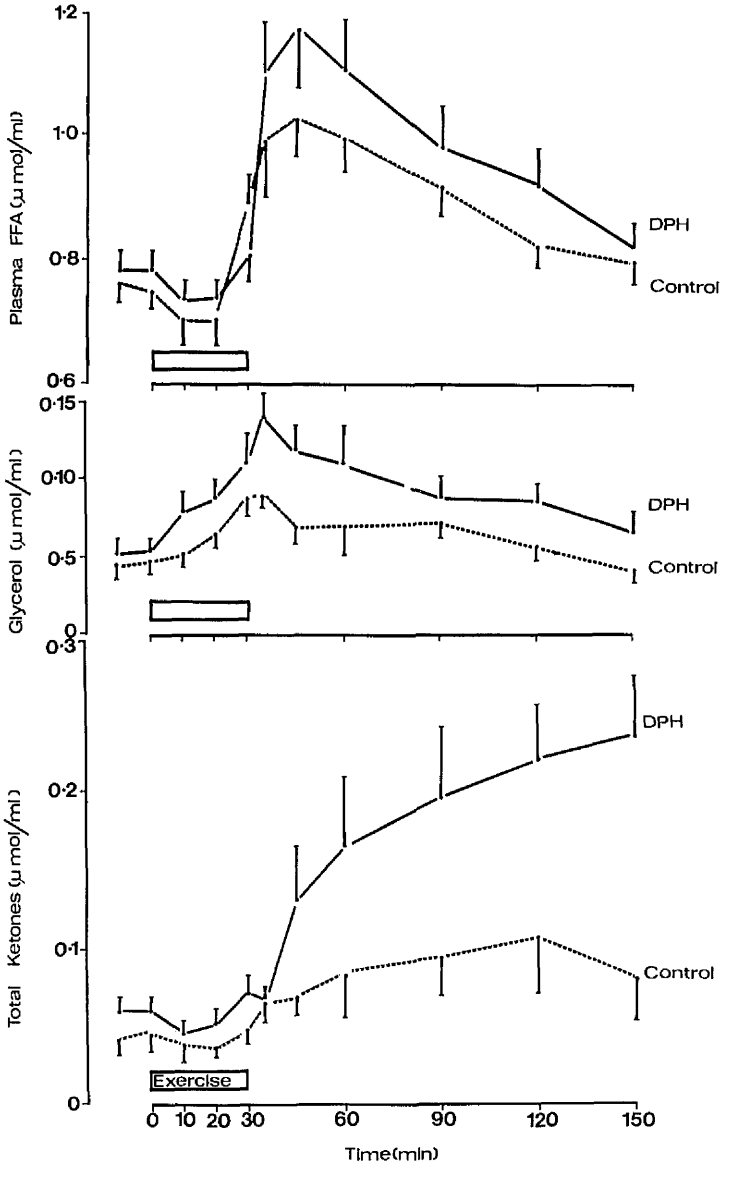
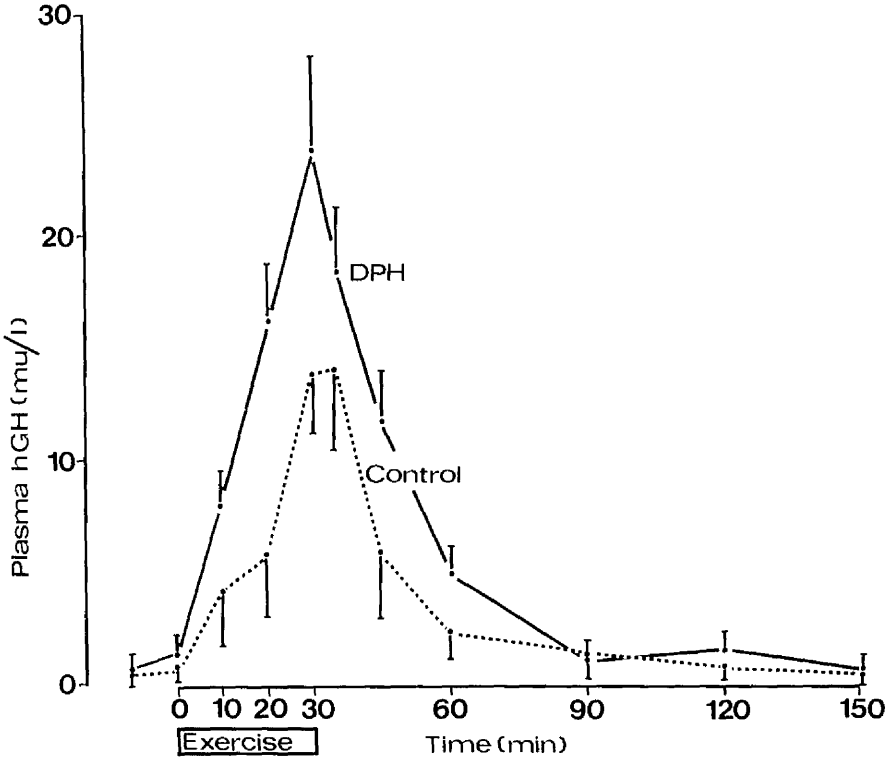

Plasma FFA (μ mol/ml)
1·2
1·0
0·8
0·6
DPH
Control
Glycerol (μ mol/ml)
0·15
0·10
0·5
0
DPH
Control
Total Ketones (μ mol/ml)
0·3
0·2
0·1
0
DPH
Control
Exercise
0 10 20 30 60 90 120 150
Time(min)

FIG 1.II Plasma hGH concentrations (mu/l, mean $\pm$ SEM) during and after exercise in six normal subjects with (●——●) and without (●----●) administration of DPH (500 mg).


30
20
10
0
Plasma hGH (mu/l)
DPH
Control
0 10 20 30 60 90 120 150
Exercise
Time (min)

DISCUSSION

The results presented in this chapter demonstrate that administration of DPH to normal subjects alters metabolic and hGH responses to exercise. The observation of a greater rise in FFA and blood glycerol during and initially after exercise suggests that DPH produces greater lipolysis during exercise. The development of marked post exercise ketosis was also noted following DPH treatment.

The factors responsible for the increased lipolysis associated with DPH treatment are unknown. Moderate exercise has been shown to cause a rise in plasma cortisol as exercise continues (Sutton, Young, Lazarus, Hickie and Maskvytis, 1968) and DPH stimulates hydroxycorticoid excretion (Costa et al., 1955; Krieger, 1962; Dill, 1966). Cortisol inhibits FFA re-esetrification and potentiates FFA release by adrenaline, (Shafrir and Kerpel, 1969). Plasma catecholamine concentrations are reported to increase with exercise (Bloom, Johnson, Park, Rennie and Sulaiman, 1976) and catecholamines promote lipolysis (Havel and Goldfien, 1959). It is not known if changes in circulating levels of cortisol or catecholamines were important factors leading to increased lipolysis after DPH treatment in the present study and this requires further investigation. The present observations did not demonstrate any increased lipolysis at rest before exercise suggesting that the drug potentiates responses to exercise.

Ketone concentrations did not alter greatly during exercise on both occasions suggesting that ketone production and utilization was closely matched during exercise. Treatment with DPH was associated with a marked rise in ketones after exercise. The greater rise in ketone concentrations is probably related to the greater lipolysis during and initially after exercise combined with a decreased aerobic oxidation through the citric acid cycle. This effect may have been produced by a reduced rate of re-esterification of FFA after exercise. Insulin levels are known to fall during exercise and to rise immediately after exercise paralleling changes in adrenergic activity (Johnson, Park, Rennie and Sulaiman, 1974). A failure of release of insulin after exercise in the subjects treated with DPH would be consistent with the known effects of DPH in inhibiting insulin release (Malherbe et al., 1972; Levin et al., 1970) and such an effect could delay re-esterification of FFA as insulin is known to limit fat mobilization (Bieberdorf, Chernick and Scow, 1970).

The present observations indicate that DPH treatment produces a greater hGH response to exercise. Growth hormone is known to have lipolytic properties (Hunter, Fonseka and Passmore, 1965b; Rabinowitz et al., 1965) but the absence of an increase in hGH during exercise in patients with hypopituitarism did not prevent fat mobilization during exercise

(Johnson, Rennie, Walton and Webster, 1971). It therefore seems unlikely that the greater elevation of hGH produced the greater level of lipolysis during and initially after exercise in the present investigation. Hypoglycaemia (Roth et al., 1963) and a fall in FFA (Irie et al., 1967) have been reported to stimulate hGH release. Blood glucose concentrations were similar in the present investigation on both occasions during and after exercise and FFA concentrations increased in a similar manner during exercise on both occasions. The results presented in this chapter suggest that DPH stimulates hypothalamic/pituitary mechanisms regulating hGH release without affecting known metabolic stimuli.

There is evidence that hGH release during exercise is controlled by adrenergic and serotonergic mechanisms (Blackard and Heidingsfelder, 1968; Smythe and Lazarus, 1974). Treatment with DPH is known to elevate brain serotonin levels (Bonnycastle, Paasonen and Giarman, 1956) and it is possible that this effect may be the basis for the enhancedhGH response to exercise after DPH treatment in the present investigation.

The results reported in this chapter demonstrate that administration of DPH increases lipolysis during and initially after exercise and is associated with marked post exercise ketosis. DPH produced greater elevation of hGH in response to

exercise suggesting that the drug stimulates the hypothalamic/pituitary axis. Further studies of the mechanism by which DPH alters lipolysis and hGH release are indicated as these metabolic and hormonal effects may have important implications for treatment with this drug.

SUMMARY

1. Metabolic and hGH responses to exercise were investigated in six normal healthy subjects on two occasions with and without an oral dose of DPH (500 mg).

2. Serum DPH concentrations were similar in all the subjects and were just below the accepted therapeutic range for epileptic patients.

3. There was no significant difference in blood lactate, pyruvate or glucose concentrations associated with DPH treatment. Plasma FFA and blood glycerol concentrations were greater during and initially after exercise and the concentrations of total ketones were also greater after exercise following DPH treatment.

4. Treatment with DPH was associated with significantly greater concentrations of hGH during exercise and the results suggest that DPH has a stimulating action on hypothalamic/pituitary function.

5. Further investigation of the mechanisms by which DPH alters lipolysis and hGH release would be of value as these metabolic and hormonal effects may have important implications for treatment with this drug.

CHAPTER 2

THE EFFECT OF DIPHENYLHYDANTOIN ON GLUCOSE TOLERANCE IN EPILEPTICS

INTRODUCTION

Diphenylhydantoin (DPH) is known to produce hyperglycaemia in animals (Belton, Etheridge and Millichap, 1965; Sanbar, Conway, Sweifler and Smet, 1967) and in patients treated with high doses of DPH intravenously for epilepsy or neurological disorders (Klein, 1966; Goldberg and Sanbar, 1969). Other studies have demonstrated that DPH impairs insulin release in response to glucose loading (Malherbe et al., 1972) and inhibits insulin release from isolated rat pancreas 'in vitro' (Levin et al., 1970). Studies of DPH induced hyperglycaemia have in volved administration of relatively high doses of DPH and it is not clear if hyperglycaemia or impaired insulin release are important side effects of long term treatment with therapeutic doses of this drug. It was decided to investigate the possible importance of this effect by studying glucose tolerance in a group of chronic epileptic patients on long term treatment with DPH.

METHODS

Patients and Subjects

Six patients resident in an epilepsy centre (Quarrier's Homes, Bridge of Weir) were studied. The patients, three males and three females (aged 29-40 years) were all receiving long term treatment with DPH (200-500 mg/day) and had received similar doses of this drug for not less than five years.

None of the patients were known to be diabetic and they all gave their consent to take part in the investigation. In addition six normal healthy control subjects, three males and three females (aged 30-52 years) volunteered for the investigation.

Procedure

Blood samples were withdrawn from a cannula in an antecubital vein at rest and at 30, 60, 90, 120 and 150 minutes after taking 50g of glucose in 200 ml of water orally. Resting serum samples were analysed for DPH and plasma samples analysed for hGH and insulin. Blood samples were assayed for glucose. Significance of differences were examined using the Mann-Whitney non-parametric U-test for small samples (Mann and Whitney, 1947).

RESULTS

Serum diphenylhydantoin (Table 2.I)

Serum DPH concentrations were similar in the six patients and were all within the accepted therapeutic range of 40-80 μmol/l (Lancet, 1975).

Blood glucose (Fig 2.I)

There was no difference between basal blood glucose concentrations between patients and controls. After the administration of glucose, blood glucose concentration rose in both groups but the concentrations in the patients were significantly greater than the controls at 30, 60, 90 and 120 minutes ($P<0.05$, 0.01, 0.05 and 0.05 respectively).

Plasma insulin (Fig 2.I)

There was no significant difference in basal insulin concentrations between the two groups. After glucose administration insulin concentrations rose in both groups and reached peak concentration in the controls 30 minutes after glucose whereas in the patients the peak concentration occurred at 60 minutes. The insulin concentrations in the patients were significantly lower than the controls at 30 minutes ($P<0.05$).

Plasma growth hormone

There was no significant difference in basal hGH

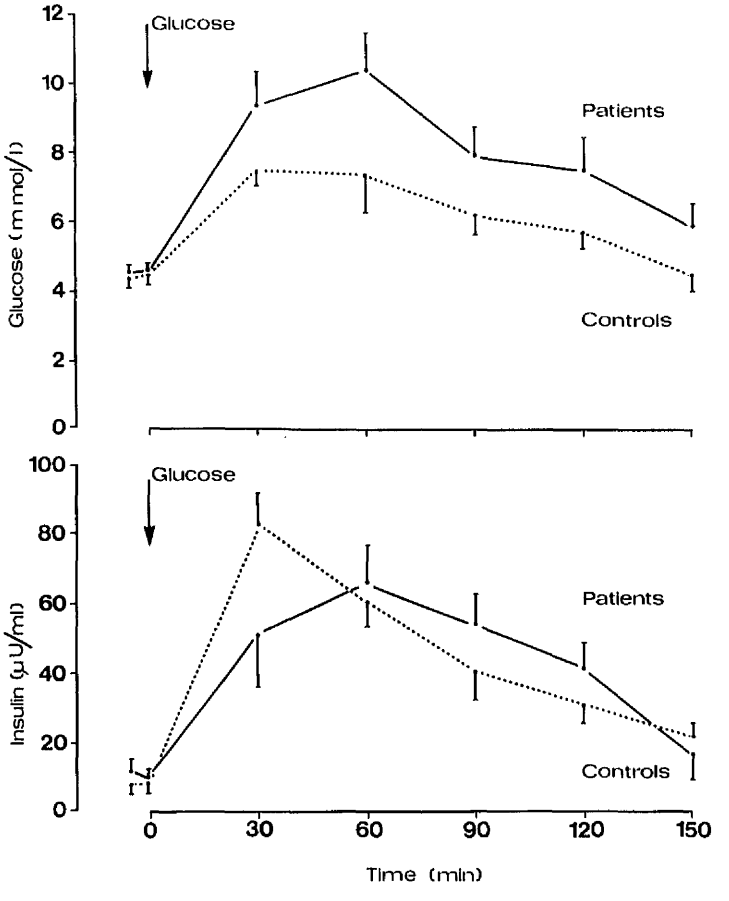
concentrations between the two groups. Both groups showed a similar depression in hGH concentrations after oral glucose and there was no significant difference in hGH concentrations between the groups at any time during the investigation.

TABLE 2.I

Serum DPH concentrations in six epileptics before a glucose tolerance test.

Patient No.	Age Years	Sex	Serum DPH μmol/l
1	40	M	59.2
2	33	F	72.8
3	34	M	46.4
4	31	M	67.2
5	29	M	81.6
6	32	F	61.2

FIG 2.I Blood glucose (mmol/l, mean ± SEM) and plasma insulin concentrations (μU/ml, mean ± SEM) in six epileptic patients treated with DPH (●——●) and in six healthy control subjects (●- - - - - - - - -●).


Glucose
Patients
Controls
Glucose (mmol/l)
12
10
8
6
4
2
0
Glucose
Patients
Controls
Insulin (μU/ml)
100
80
60
40
20
0
0
30
60
90
120
150
Time (min)

DISCUSSION

The results reported in this chapter demonstrate an impaired glucose tolerance in epileptic patients receiving long term treatment with DPH. Plasma insulin concentrations increased in response to glucose in all the patients but the rate of increase was slower and the peak insulin concentrations were lower than the controls. The results suggest that the impaired glucose tolerance was at least partly due to a reduced insulin response.

Studies of glucose tolerance in young epileptics treated with DPH (Cummings, Rosenbloom, Kohler and Wilder, 1973) and in six chronic epileptics receiving long term treatment with DPH (Castleden and Richens, 1973) have failed to demonstrate any impairment of glucose tolerance or insulin release. The hyperglycaemia effect of DPH has been reported to be related to the amount of drug administered (Faris and Lutcher, 1971). Castleden and Richens (1973) reported that their patients had serum DPH concentrations of 25-125 μmol/l which is similar to the range of concentrations found in the patients in the present investigation and the impaired glucose tolerance found in the patients in the present study was not therefore associated with greater serum DPH concentrations. Elevated growth hormone levels are known to impair glucose utilisation (Luft and Cerasi, 1964) but basal hGH concentrations were not elevated in the patients in the present study and hGH

concentrations showed the normal suppression following glucose loading (Roth, Glick, Yallow and Berson, 1963).

The results presented in this chapter demonstrate impaired glucose tolerance and a reduced insulin response in patients receiving long term treatment with DPH. These results are not in agreement with other studies of glucose tolerance in DPH treated epileptics but the alteration of glucose tolerance and the insulin response was relatively small and this suggests that hyperglycaemia and impaired insulin release are not important side effects of long term treatment with therapeutic doses of DPH.

SUMMARY

1. Glucose tolerance was investigated in six epileptic patients receiving long term treatment with DPH (200-500 mg/day).

2. Serum DPH concentrations were all within the accepted therapeutic range (40-80 μmol/l).

3. Blood glucose concentration in response to glucose loading were significantly greater than controls and insulin concentrations were significantly lower than controls.

4. The impairment of glucose tolerance and the reduction of the insulin response were both relatively small in the patients and this suggests that hyperglycaemia and impaired insulin release are not important side effects of long term treatment with therapeutic doses of DPH.

CHAPTER 3

THE EFFECT OF ALCOHOL ON GROWTH HORMONE AND PROLACTIN CONCENTRATIONS AT REST AND IN RESPONSE TO EXERCISE

INTRODUCTION

Alcohol is known to produce a number of changes in endocrine functions (Gordon and Southern, 1977). Reduced growth has been demonstrated in young animals treated with alcohol compared with isocalorically matched controls (Leiber, Jones and De Carli, 1965). Growth retardation has been attributed to energy wastage since microsomal ethanol metabolism generates no high energy bonds and energy is dissipated as heat (Leiber, 1973). However, there have been no studies of growth hormone in relation to growth retardation by alcohol and the possibility exists that alcohol affects hypothalamic/pituitary function and alters growth hormone release. An action of alcohol on the hypothalamic/pituitary axis is suggested by the reports that administration of alcohol is associated with a rise in cortisol concentrations in normal subjects (Merry and Marks, 1969; Bellet et al., 1970) and that this response is absent in patients with pituitary adenomas (Jenkins and Connolly, 1968). In addition, increased hGH release is reported to follow administration of ethanol (1.5 ml/kg body weight) (Bellet et al., 1971). Studies of plasma concentrations of hGH and hPRL may be of value in determining if alcohol has an effect on hypothalamic/pituitary function. It was therefore decided to investigate the effect of alcohol on hGH and hPRL levels at rest and in response to exercise. The results of these investigations are presented and discussed in this chapter.

I. INVESTIGATIONS AT REST

Subjects

Five normal healthy male subjects, mean age 28 years (range 22-35 years) were studied. The subjects were investigated in the morning following an overnight fast and remained at rest sitting or lying down throughout the investigation.

Procedure

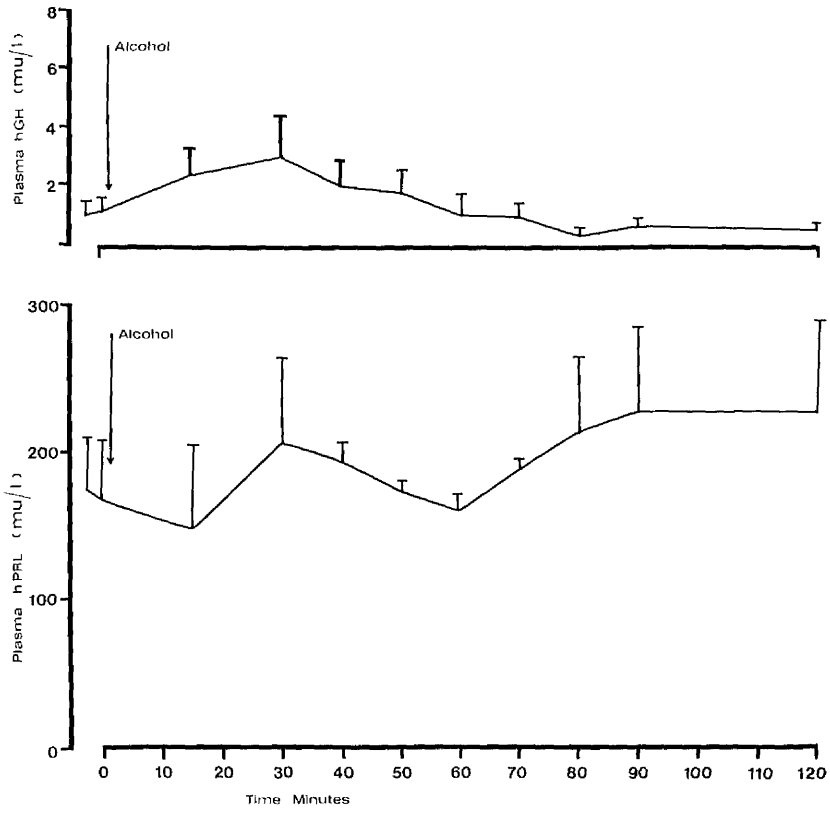
Blood samples were taken from a cannula in an antecubital vein before and at 15, 30, 40, 50, 60, 70, 80, 90 and 12 minutes after they had taken a drink containing ethanol (0.5g/kg body weight) made up to 200 ml with water. Plasma samples were assayed for hGH and hPRL.

RESULTS

Plasma hGH and hPRL (Fig. 3.I)

There was no significant change from basal hGH or hPRL concentrations after the ingestion of alcohol.

FIG. 3.I Plasma growth hormone and prolactin concentrations (mu/l, mean $\pm$ SEM) before and after oral administration of alcohol (0.5g/kg body weight) in five normal healthy male subjects.


Plasma hGH (mu/l)
8
6
4
2
Alcohol
Plasma hPRL (mu/l)
300
200
100
0
Alcohol
0
10
20
30
40
50
60
70
80
90
100
110
120
Time Minutes

II. INVESTIGATIONS DURING AND AFTER EXERCISE

Subjects

Five normal healthy male subjects aged 22-35 years (mean age 28 years) were investigated in the morning following an overnight fast.

Procedure

The subjects performed exercise on a bicycle ergometer at 600 kpm for 30 minutes on two occasions with and without an oral dose of alcohol (0.5g/kg body weight). Blood samples were taken from a cannula in an antecubital vein before, at five minute intervals during exercise and at 5, 15, 30, 45, 60 and 90 minutes after exercise. The exercise test commenced 15 minutes after the alcohol had been administered.

RESULTS

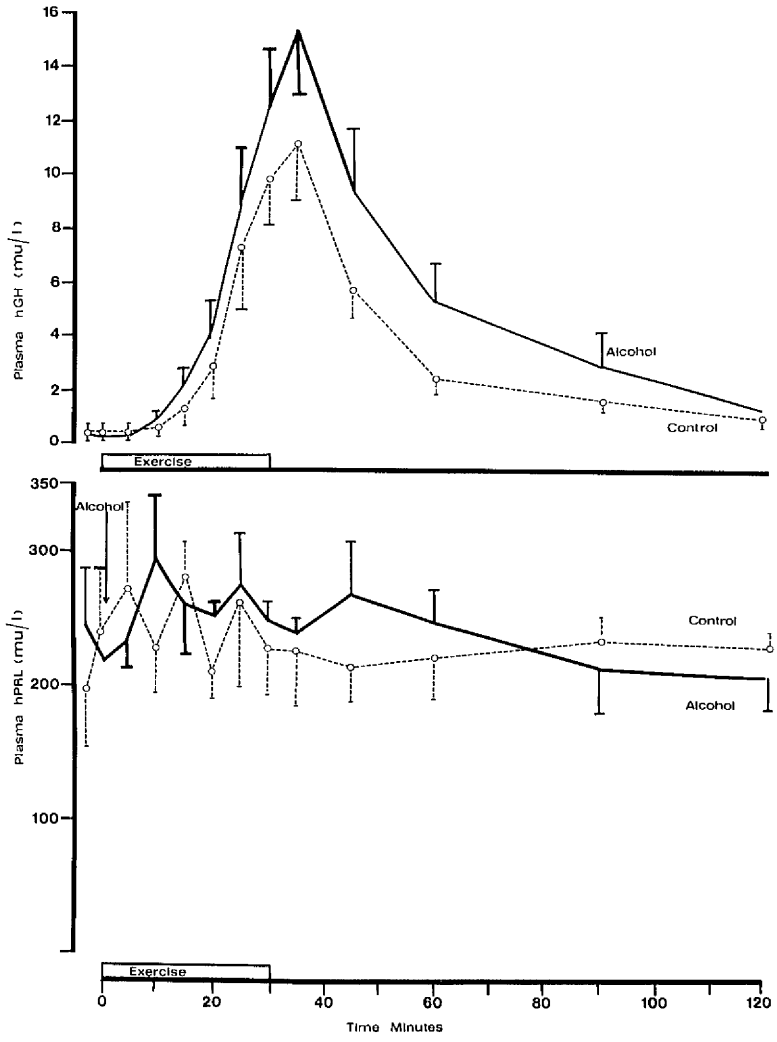
Plasma hGH (Fig 3.II)

There was no significant difference between basal hGH concentrations on the two occasions. Exercise was associated with a rise in hGH and concentrations at 35, 45 and 60 minutes were significantly greater than the corresponding control values ($P < 0.01$, 0.05 and 0.02 respectively).

Plasma hPRL (Fig 3.II)

Basal hPRL concentrations were not significantly different on the two occasions. Exercise was not associated with any significant change in hPRL concentrations on either occasion and there was no significant difference in hPRL concentrations on the two occasions at any time during the investigation.

FIG. 3.II Plasma growth hormone and prolactin concentrations (mu/l, mean ± SEM) during and after exercise in five normal healthy male subjects with (o——o) and without (●----●) an oral dose of alcohol (0.5g/kg body weight).


16
14
12
10
8
6
4
2
0
Plasma nGH (mu/l)
Alcohol
Control
Exercise
350
300
200
100
Plasma hPRL (mu/l)
Alcohol
Control
Alcohol
Exercise
0
20
40
60
80
100
120
Time Minutes

DISCUSSION

The results presented in this chapter demonstrate that alcohol (0.5g/kg body weight) had no effect on hGH or hPRL concentrations at rest or on hPRL levels during exercise but do demonstrate that alcohol significantly increased the hGH response to exercise. The failure to find a change in hGH and hPRL concentrations after alcohol at rest is consistent with the reported absence of any response to 1g/kg body weight (Toro, Kolodny, Masters and Daughaday, 1973), but is not in agreement with the finding of an elevation of hGH in response to 1.5 ml/kg body weight (Bellet et al., 1970). Bellet and co-workers reported blood alcohol levels up to 140mg/100ml in their study whereas 0.5g/kg body weight is known to produce blood alcohol levels of only 40mg/100ml (Chalmers, 1976). The possibility therefore exists that the dose of alcohol used in the present study did not produce sufficient elevation of blood alcohol levels to produce an effect on hGH release at rest.

The increased hGH response to exercise associated with alcohol in the present study was not due simply to elevation of resting hGH levels and the change in hGH levels appeared to be independent of any change in hPRL levels. The alteration of the hGH response may be due to some effect of alcohol on metabolic stimuli or release mechanisms. Growth

hormone is known to be released in response to a fall in blood glucose (Hunter, Fonseka and Passmore, 1965a) or FFA (Hartog, Havel, Copinschi, Earll and Ritchie, 1967) and to a rise in blood lactate (Sutton, Young, Lazarus, Hickie and Maskvytis, 1968). The effect of alcohol (0.5g/kg body weight) on metabolic changes during exercise has been the subject of earlier investigations and alcohol was not found to be associated with a greater elevation of blood lactate during exercise, or a significant change in blood glucose but was associated with increased levels of glycerol and FFA during exercise (Chalmers, 1976). It therefore appears unlikely that the greater hGH response found in the present investigation is due to an effect of alcohol on glucose, lactate or FFA concentrations during exercise. It is possible that the greater elevation of hGH was due to a decrease in peripheral hGH uptake or metabolism but there was no evidence for this in the studies at rest. The results presented in this chapter suggest that alcohol potentiates the hGH response to exercise and that this effect is not related to a change in metabolic stimuli for hGH release.

The hGH response to arginine has been reported to be reduced by alcohol infusion (75g alcohol over 4 hours) and it has been suggested that this may be due to an action of alcohol on the hypothalamus (Tamburrano, Tamburrano, Gambardella and Andreani, 1976). Although this result is

not consistent with the present findings of an enhanced hGH response to exercise this may reflect a difference in the regulation of release of hGH to these different stimuli. Administration of the α-adrenergic receptor blocking agent, phentolamine, blunts the hGH response to exercise (Hansen, 1971) but the reports of the effect of this drug on the arginine induced hGH response are not consistent, (Strauch, Modigliani and Bricaine, 1968; Buckler, Bold, Taberner and London, 1969; Lufkin, Greene and Meek, 1971). The anti-serotinergic drug, cyproheptadine, has been shown to reduce both the hGH response to exercise and to arginine (Smythe and Lazarus, 1974; Nakai et al., 1973), and this suggests that serotonin is involved in regulating hGH release to both these stimuli. Although the regulating mechanism for these hGH responses are not clearly established the evidence does suggest that the exercise response involves adrenergic and serotonergic mechanisms whereas for the arginine response only serotonergic mechanisms have been established. It is possible that the different effects of alcohol on the hGH responses to exercise and arginine are related to differences in the regulation of release of hGH to these stimuli but this suggestion requires further investigation.

Studies of the effects of alcohol on brain serotonin are not consistent and acute alcohol administration does not significantly alter steady state levels of brain noradrenaline

or dopamine (Noble and Tewari, 1977). However, there is evidence that alcohol alters brain amine turnover (Corrodi, Fuxe and Hokfelt, 1966) and the possibility that the alteration of hGH responses to exercise and arginine by alcohol is related to an effect on hypothalmic catecholamine turnover requires further study. The results presented in this chapter demonstrate that alcohol alters hGH release in normal subjects and it was decided to investigate hGH responses in chronic alcoholics to determine if there is any evidence for altered hGH release following chronic alcohol abuse. The results of a number of studies in patients with a progressive alcohol problem are presented in the next three chapters.

SUMMARY

1. The effect of oral administration of alcohol (0.5 g/kg body weight) on basal hGH and hPRL concentrations was investigated in five normal healthy subjects. In addition, concentrations of hGH and hPRL were investigated during and after exercise in five healthy subjects on two occasions with and without an oral dose of alcohol (0.5 g/kg body weight).

2. Alcohol administration did not significantly alter basal concentrations of hGH or hPRL.

3. Growth hormone concentrations were significantly greater during exercise after alcohol administration than in the control investigation. Concentrations of hPRL during and after exercise were not significantly altered by alcohol.

4. The results suggest that alcohol potentiates hGH release in response to exercise. This effect does not appear to be associated with a fall in blood glucose or FFA concentrations and may be related to a direct effect of alcohol on hGH release mechanisms.

CHAPTER 4

THE METABOLIC RESPONSE TO EXERCISE IN CHRONIC ALCOHOLICS

INTRODUCTION

Moderate exercise produces increased levels of human growth hormone in the blood (Hunter et al., 1965a) and the changes in certain metabolites with exercise give an indication of carbohydrate and fat metabolism. Acute ingestion of ethanol can also lead to increased secretion of human growth hormone (Bellet et al., 1971) and affects carbohydrate metabolism (Hawkins and Kalant, 1972). Patients with a history of chronically high alcohol intake have a greater decrease in muscle glycogen during exercise than normal subjects (Suominen, Forsberg, Heikkinen and Osterback, 1974). There is evidence that some chronic alcoholics have a depression of their hypothalamic/pituitary/adrenal axis as their cortisol response to oral ingestion of ethanol is absent (Merry and Marks, 1969). In order to investigate metabolic and hormonal responses to exercise in chronic alcoholism six subjects were studied within a few days of admission for management of this problem. They had not ingested alcohol in the intervening time.

METHODS

Subjects

Six male subjects (age 40-48 years) who had a progressive alcohol problem of not less than six years duration were

studied. Alcohol abuse had been so persistent that it had affected their everyday lives (Table 4.I). They had a mean height of 173 cm $\pm$ 3.0 (S.E.M.) and a mean weight of 72.2 kg $\pm$ 3.2 (S.E.M.). Six healthy male subjects (age 26-40 years) were also examined as controls. They were all members of the University Department of Neurology, had no admitted alcohol problem or evidence of it, and to the best of my knowledge had abstained from alcohol for the previous 24 hours. They had a mean height of 176 cm $\pm$ 3.0 (S.E.M.) and a mean weight of 74.2 kg $\pm$ 3.6 (S.E.M.). The patients were investigated after they had been admitted to a psychiatric unit for treatment of their chronic alcoholism. None of the patients had taken alcohol since admission 9-16 days earlier and liver function tests were all normal.

Procedure

After an overnight fast, patients and control subjects performed exercise on a bicycle ergometer (Elema Schonander constant load ergometer EM 369) for 20 minutes at a fixed work load of 100 watts. Heart rate was recorded during exercise and for 5 minutes after exercise using an electrocardiograph. A catheter was placed in a forearm vein and blood samples were taken before exercise, at five minute intervals during exercise and then at 15, 30, 60 and 90 minutes after exercise. Blood samples were analysed for glucose, lactate, pyruvate, glycerol, acetoacetate and 3-hydroxybutyrate.

Plasma samples were analysed for free fatty acids (FFA) and for human growth hormone (hGH). Significances of differences were examined with the Mann-Whitney non-parametric U test for small samples (Mann and Whitney, 1947).

TABLE 4.I Clinical symptoms and features of history associated with prolonged alcohol abuse in six alcoholics.

Admitted alcohol intake (g alcohol/week)		Duration of alcohol problem (years)		Number of patients with particular symptoms or features present during history				
mean	range	mean	range	tremor	delirium tremens	cognitive impairment	work and family problems	recent arrest
1299	(585-1735)	15	(6-30)	6	6	6	3	3

RESULTS

Heart rate (Table 4.II).

The mean heart rates of the alcoholics were slightly greater both at the end of exercise and five minutes after exercise but the differences were not significant.

Blood glucose

There was no significant difference between the blood glucose of the alcoholics and the control subjects.

Blood lactate (Fig 4.I)

Both patients and subjects showed increased blood lactate concentrations during exercise and a rapid recovery to within the resting level after exercise. There was no significant difference between the resting lactate concentrations in the two groups. At the end of exercise the blood lactate of the alcoholics was significantly greater ($P < 0.01$) than the corresponding level for the control subjects.

Blood pyruvate (Fig 4.I)

There was no significant difference between the resting pyruvate concentrations of the two groups. At the end of exercise the pyruvate concentration of the alcoholics was significantly greater ($P < 0.05$) than the corresponding level for the control subjects. During the post-exercise period the blood pyruvate of the alcoholics remained significantly

TABLE 4.II Heart rate (beats.min^{-1}) during and at five minutes after exercise in six alcoholics and six control subjects

Time (min)	Alcoholics mean ± s.e.mean	Controls mean ± s.e.mean
rest	82 ± 7	78 ± 5
5	122 ± 11	120 ± 9
10	134 ± 12	128 ± 12
15	138 ± 16	134 ± 13
20	140 ± 16	136 ± 16
5 post exercise	102 ± 11	96 ± 9

greater ($P < 0.01$) than the corresponding control concentrations.

Total ketones (Fig 4.II)

There was no significant difference between the total blood ketones of the alcoholics and controls at rest. Both groups showed a similar fall in ketones during exercise. After exercise the total ketones rose in both groups, however, the concentrations reached by the alcoholics were significantly greater ($P < 0.001$) than in the controls for samples taken at 90 and 120 minutes.

Blood glycerol (Fig 4.III)

There was no significant difference between the resting glycerol concentrations in the two groups. In all samples taken during exercise the blood glycerol of the alcoholics was significantly greater ($P \leq 0.01$) than the corresponding levels for the control subjects. The only significant difference between the blood glycerol of the two groups after exercise occurred in the samples at 90 minutes when the blood glycerol of the alcoholics was significantly greater ($P < 0.05$) than the corresponding control concentration.

Plasma FFA (Fig 4.III)

The pattern of change of FFA was very similar in both the alcoholics and the control subjects. The alcoholics tended to have higher FFA levels but the difference was not significant.

Plasma hGH (Fig 4.IV)

Resting hGH concentrations were not significantly different in the two groups. Plasma hGH concentrations were lower in the alcoholics both during and after exercise but differences were significant ($P < 0.05$) only at the end of exercise and at 5 minutes after exercise.

FIG 4.I Blood lactate and pyruvate (μmol/ml, mean ± SEM) during and after twenty minutes of exercise in six chronic alcoholics (●—●) and six normal control subjects (■---■).

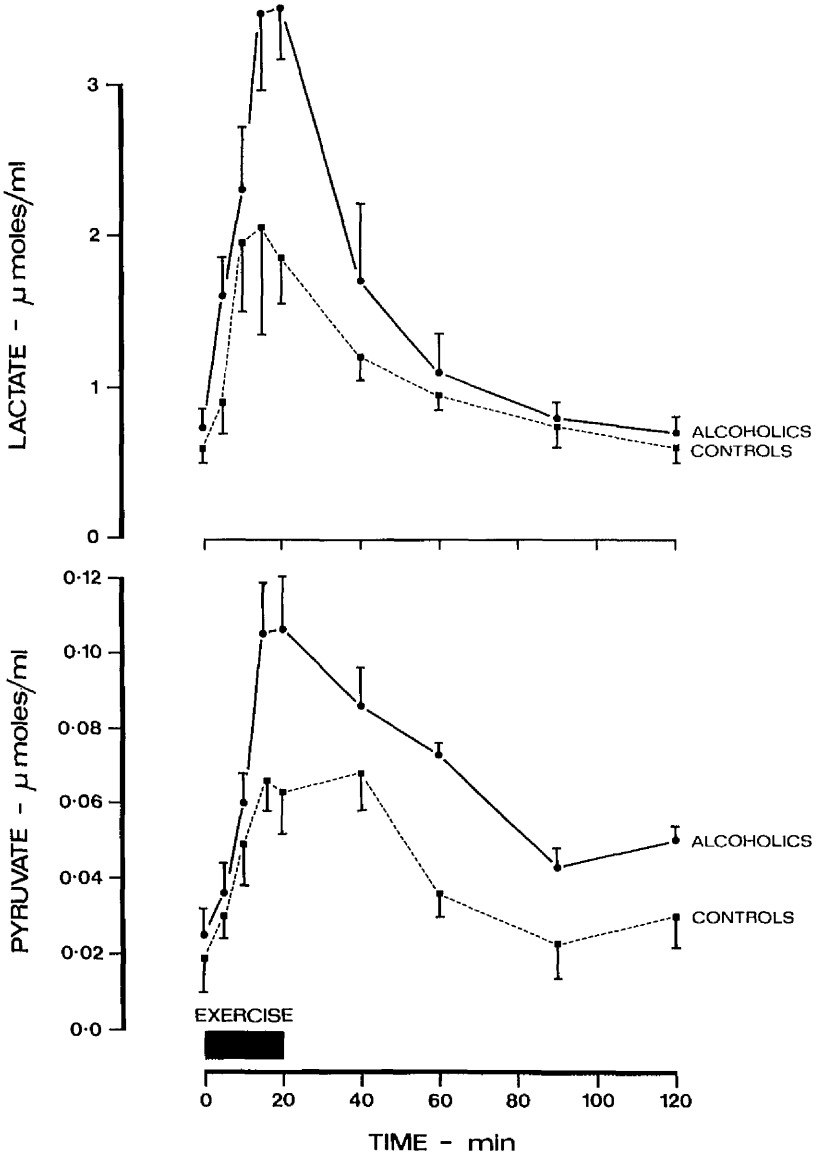
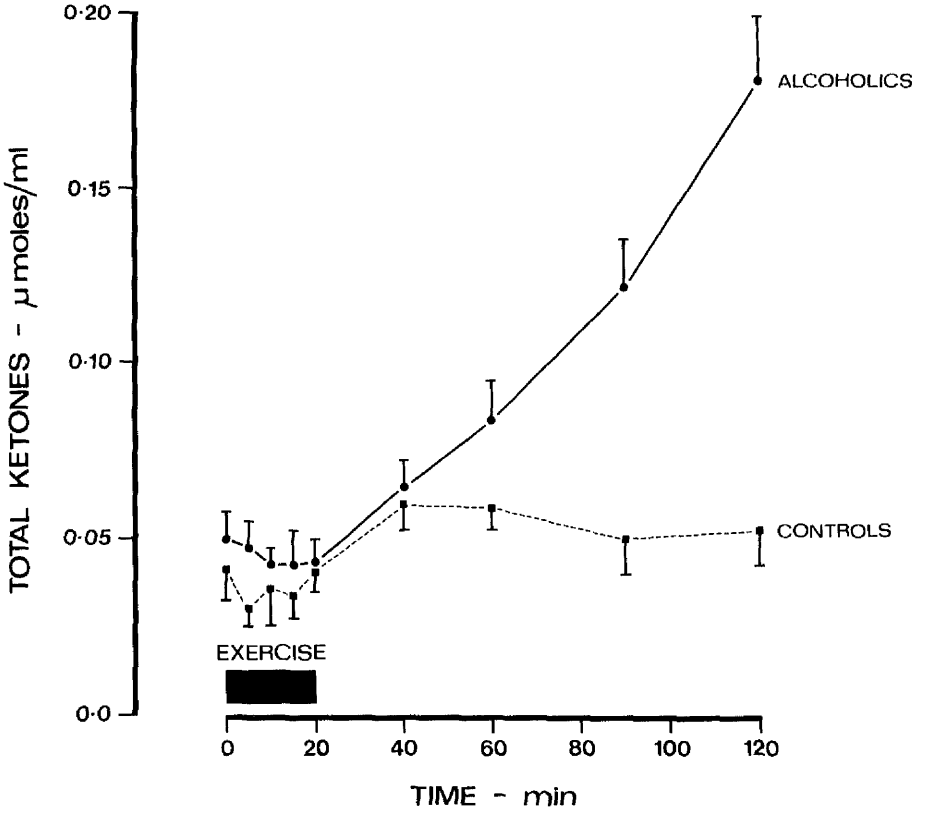

LACTATE - μ moles/ml
3
2
1
0
ALCOHOLICS
CONTROLS
PYRUVATE - μ moles/ml
0·12
0·10
0·08
0·06
0·04
0·02
0·0
ALCOHOLICS
CONTROLS
EXERCISE
0
20
40
60
80
100
120
TIME - min

FIG 4.II Total blood ketones (3-hydroxybutyrate + acetoacetate, μmol/ml mean ± S.E.M.) during and after twenty minutes of exercise in six chronic alcoholics (●——●) and six normal control subjects (■---■).

FIG 4.III Blood glycerol (μmol/ml, mean ± S.E.M.) and plasma free fatty acids (μmol/ml, mean ± S.E.M.) during and after twenty minutes of exercise in six chronic alcoholics (●——●) and six normal control subjects (■----■).


TOTAL KETONES - μ moles/ml
0·20
0·15
0·10
0·05
0·0
ALCOHOLICS
CONTROLS
EXERCISE
0
20
40
60
80
100
120
TIME - min

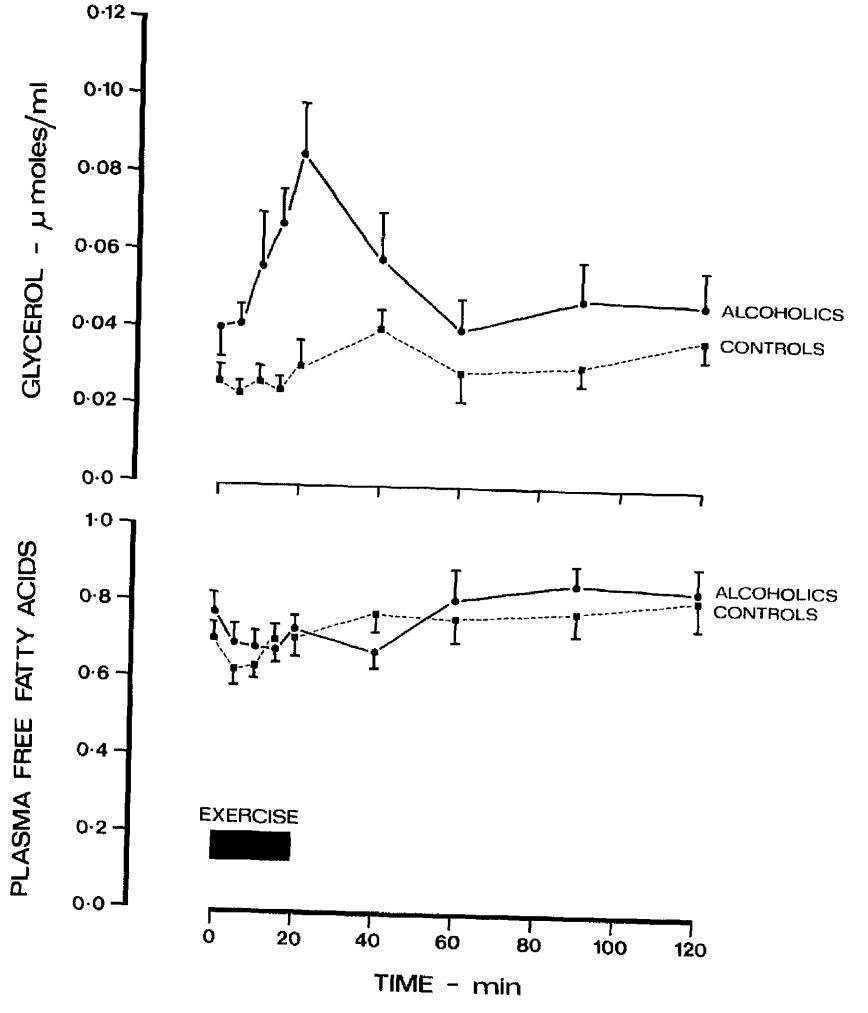
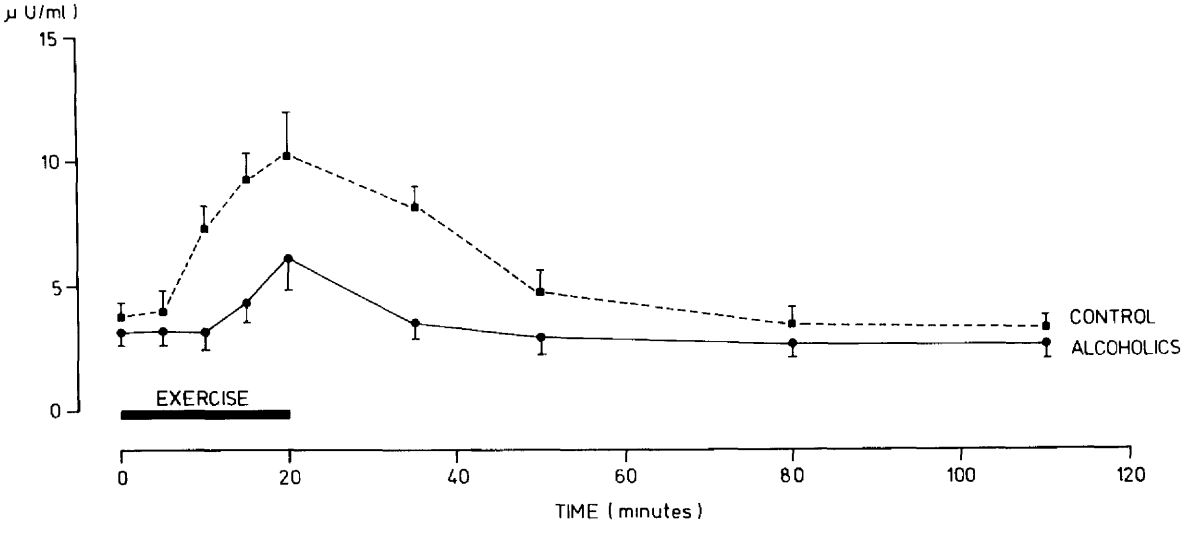

GLYCEROL - μ moles/ml
0·12
0·10
0·08
0·06
0·04
0·02
0·0
ALCOHOLICS
CONTROLS
PLASMA FREE FATTY ACIDS
1·0
0·8
0·6
0·4
0·2
0·0
ALCOHOLICS
CONTROLS
EXERCISE
0
20
40
60
80
100
120
TIME - min

FIG 4.IV Plasma hGH (μ units/ml, mean ± S.E.M.) during and after twenty minutes of exercise in six chronic alcoholics (⊙——⊙) and six normal control subjects (■----■).


μ U/ml)
15
10
5
0
EXERCISE
0
20
40
60
80
100
120
TIME (minutes)
CONTROL
ALCOHOLICS

DISCUSSION

The alcoholics performed the same exercise test as the controls and had similar heart rates but there were marked differences observed in metabolites and hormones in the blood between the two groups. The alcoholic subjects were all examined when abstinent and therefore the results are relevant to the period of withdrawal from alcohol. Further investigations will be necessary to determine whether the metabolic and hormonal changes observed in the present study are related to the chronic effect of alcohol or specifically to withdrawal. There are, however, difficulties in examining patients when under the effect of alcohol as the direct metabolic effect of alcohol would also be observed. In the present study the alcoholics were found to have significantly greater lactate and pyruvate concentrations during exercise and also developed a post-exercise ketosis. Greater concentrations of lactate and pyruvate are known to occur in untrained subjects during exercise (Robinson and Harmon, 1941; Holmgren and Strom, 1959; Cobb and Johnson, 1963; Juchems and Kumper, 1968; Saltin and Karlsson, 1971). A greater post-exercise ketosis occurs in unfit compared with fit subjects (Johnson, Walton, Krebs and Williamson, 1969; Jennett, Johnson and Rennie, 1972; Johnson and Walton, 1972). It is, therefore, possible that the alcoholics were less fit than the control subjects but this seems unlikely since the heart rate, which is known to be a good

indication of fitness, was similar in both groups at the end of exercise and during recovery.

The further possibility exists that the alcoholics had a reduced oxidative capacity not related to fitness. Muscle biopsies of alcoholics may show swollen pale muscle fibres and it is possible that such an abnormality may be a basis for reduced oxidative capacity in alcoholics but this suggestion requires further study (Klinkerfuss, Bleisch, Dioso and Perkoff, 1967). The high concentrations of glycerol in the alcoholics suggests that there was greater lipolysis during exercise in this group (Winkler, Steele and Altszuler, 1969). Fat appears to have been a more important fuel during exercise in the alcoholics but the development of post-exercise ketosis suggests that the oxidation of fat is less efficient than in the control subjects.

Exercise causes a rise in blood levels of human growth hormone and it has been suggested that growth hormone may play an important role in the initial mobilization of fat during exercise (Hunter et al., 1965b; Rabinowitz et al., 1965; Winkler et al., 1969). This suggestion has been weakened by the results of studies of patients with hypopituitarism who fail to show an increase in growth hormone during exercise

but have increased FFA levels (Johnson, Rennie, Walton and Webster, 1971). The present findings of lower human growth hormone in the alcoholics also weakens this suggestion since the levels of glycerol were greater than for the controls suggesting increased lipolysis in the alcoholics.

It has been shown that the rise of hGH with exercise is greater for untrained than for trained subjects (Hartley, Mason, Hogan, Jones, Kotchen, Mongey, Wherry, Pennington and Ricketts, 1972; Rennie and Johnson, 1974; Bloom et al., 1976). Hypoglycaemia (Hunter et al., 1965a) fall of plasma FFA (Hartog et al., 1967) and a rise of blood lactate (Sutton et al., 1968) have been reported as possible stimuli for producing an increase in plasma hGH concentration during exercise. These three possibilities appear to be unlikely in this situation, because the changes of blood glucose and plasma FFA during exercise were similar in both groups and blood lactate was higher in the patients than in the controls. In the present investigation we might have expected that if the increased levels of lactate and pyruvate during exercise and the ketosis after exercise in the alcoholics were due to lower fitness a greater, instead of a reduced, growth hormone response to exercise would have occurred.

It has been demonstrated that acute administration of alcohol (1 ml/kg body weight) in normal subjects leads to

increased cortisol levels and that this response is absent in patients with pituitary adenoma (Jenkins and Connolly, 1968). This suggests that acute intake of alcohol stimulates adrenocorticotrophic hormone (ACTH) release. Further evidence for an effect of alcohol on the pituitary comes from the observation that a single oral dose of alcohol (1.5 ml/kg body weight) can lead to increased secretion of hGH (Bellet et al., 1971).

In some chronic alcoholics, however, Merry and Marks (1969) reported that plasma cortisol concentrations were paradoxically depressed after alcohol administration. They suggested that chronically self administered alcohol has a depressor effect on cortisol release via the hypothalamic-pituitary axis. It is possible that such a depressor effect may also account for the lower hGH response to exercise in the alcoholics studied in this chapter.

SUMMARY

1. The metabolic response to steady exercise was studied in six chronic alcoholics and six normal control subjects.

2. Higher concentrations of lactate and pyruvate were observed in the alcoholics during exercise and they also developed post-exercise ketosis. These changes were probably not due to reduced fitness of the alcoholics as the heart rates of both groups were similar.

3. The alcoholics had lower levels of growth hormone during exercise compared with the controls suggesting that chronic alcohol consumption has a depressor effect on pathways regulating the release of growth hormone.

CHAPTER 5

THE GROWTH HORMONE RESPONSE TO INSULIN INDUCED HYPOGLYCAEMIA IN ALCOHOLICS

INTRODUCTION

Chronic alcohol consumption may be associated with a depression of hypothalamic/pituitary/adrenal function since it has been observed that the normal rise in cortisol concentrations in response to oral alcohol and hypoglycaemia is absent in a number of alcoholics (Merry & Marks, 1972). If hypothalamic/pituitary function is depressed in alcoholics the hGH response to various hGH releasing stimuli may be impaired. Results presented in the previous chapter demonstrate that the hGH response to exercise is impaired in alcoholics. The hGH response to exercise is, however, known to vary according to work rate and fitness (Bloom et al., 1976) and it was therefore decided to investigate the hGH response to hypoglycaemia in alcoholics.

METHOD

Subjects

The growth hormone response to insulin hypoglycaemia was studied in 7 male alcoholics, mean age 42 years (range 32-51) who had given their informed consent to the investigation and 10 normal control subjects

(5 male, 5 female) mean age 42 years (32-51). In addition the cortisol response was studied in 6 male control subjects, mean age 38 years (23-51). The patients all admitted consuming relatively large amounts of alcohol on a regular basis for not less than 5 years and their everyday life had been affected (Table 5.I). Liver function tests and blood alcohol levels on admission to hospital suggest that the patients had been drinking but do not demonstrate marked liver damage. The patients had all stopped drinking 2-7 days (range) before the investigation. The control subjects were all normal volunteers who had no admitted alcohol problem.

Procedure

Blood samples were withdrawn from a cannula in an antecubital vein before and at 10, 20, 25, 30, 35, 40, 45 and 60 minutes after the injection via the cannula of soluble insulin (0.1 U/kg body weight). Blood samples were analysed for glucose and plasma samples for hGH and corticosteroids. Significance of difference was examined with the Mann-Whitney non-parametric U test for small samples (Mann & Whitney, 1947).

TABLE 5.1 Clinical and laboratory data in 7 male alcoholics

	Clinical history						Laboratory results			
Patients	Alcohol consumption g alcohol/ week	DTs	Cognitive impairment	Work and family problem	Recent arrest	Duration of alcohol problem (years)	glutamyl transpeptidase IU/l (normal up to 45)	Transaminase IU/l ALT (normal 12-40)	Transaminase IU/l AST (normal 8-47)	Blood alcohol mg/100 ml
R.A.	1638	+	-	-	-	12	87	23	50	-
T.B.	1680	-	-	+	+	7	81	25	30	-
T.C.	1680	-	-	+	-	7	45	20	21	-
J.L.	1610	-	-	+	-	10	29	65	164	122
R.S.	1260	+	-	+	-	5	55	52	74	31
J.S.	840	-	+	+	-	7	36	94	40	-
J.D.	2520	-	-	+	+	10	317	93	106	-

RESULTS

Blood glucose (Table 5.II)

The rate of fall of blood glucose after insulin injection was similar in both groups and there was no significant difference in the concentrations of the 2 groups at any time during the investigation. All the subjects developed adequate hypoglycaemia (blood glucose < 2.2 mmol/l) for the hGH response.

Plasma free fatty acids (Fig. 5.I)

Insulin produced a similar depression in FFA concentrations in both groups and there was no significant difference in the concentrations of the 2 groups before or after the injection.

Plasma growth hormone (Table 5.III)

The concentrations of hGH before and at 10 and 20 minutes after insulin injection in the alcoholics were significantly lower than the corresponding values for the controls. The mean hGH concentrations of both groups showed an increase 30-35 minutes after the injection and the alcoholics had significantly lower hGH concentrations at 45 and 60 minutes than the controls. Three of the alcoholics had an inadequate hGH response with a peak concentration less than 10 mu/l.

Plasma cortisol (Table 5.IV)

After the injection of insulin plasma cortisol concentrations increased in both groups but the concentrations in the alcoholics were significantly lower at 40, 45 and 60 minutes. Only 2 of the patients had a cortisol response greater than 200 mmol/ and cortisol concentrations decreased after insulin in 2 patients.

TABLE 5. II Blood glucose concentrations in mmol/l before and after the injection of insulin (0.1 U/kg body weight) in 7 alcoholics and the mean concentrations in 10 control subjects.

	Time (min)									
Patients	0	0	10	20	25	30	35	40	45	60
R.A.	4.4	4.5	2.3	2.7	1.8	1.8	1.9	2.2	2.6	3.8
T.B.	4.2	4.2	2.8	1.1	1.2	1.7	2.2	2.3	2.5	3.0
T.C.	4.3	4.2	3.2	2.4	1.4	1.6	1.8	2.4	3.1	3.7
J.L.	4.2	4.7	2.9	1.8	1.4	1.2	1.7	2.2	2.1	3.0
A.S.	5.0	5.1	3.6	1.8	1.0	1.1	1.2	1.4	1.6	2.8
J.S.	4.7	5.1	4.1	2.6	1.1	2.0	2.6	2.5	3.5	4.1
J.D.	4.8	4.1	2.7	2.1	1.5	1.8	2.2	2.4	3.2	3.0
Mean	4.5	4.6	3.1	2.1	1.3	1.6	1.9	2.2	2.7	3.3
S.E.M.	0.2	0.2	0.2	0.2	0.1	0.1	0.2	0.1	0.3	0.3
Controls										
Mean	4.2	4.2	3.5	2.2	1.3	1.5	1.2	1.4	2.1	2.2
S.E.M.	0.3	0.3	0.4	0.3	0.2	0.2	0.2	0.2	0.5	0.7
Significance of difference	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS

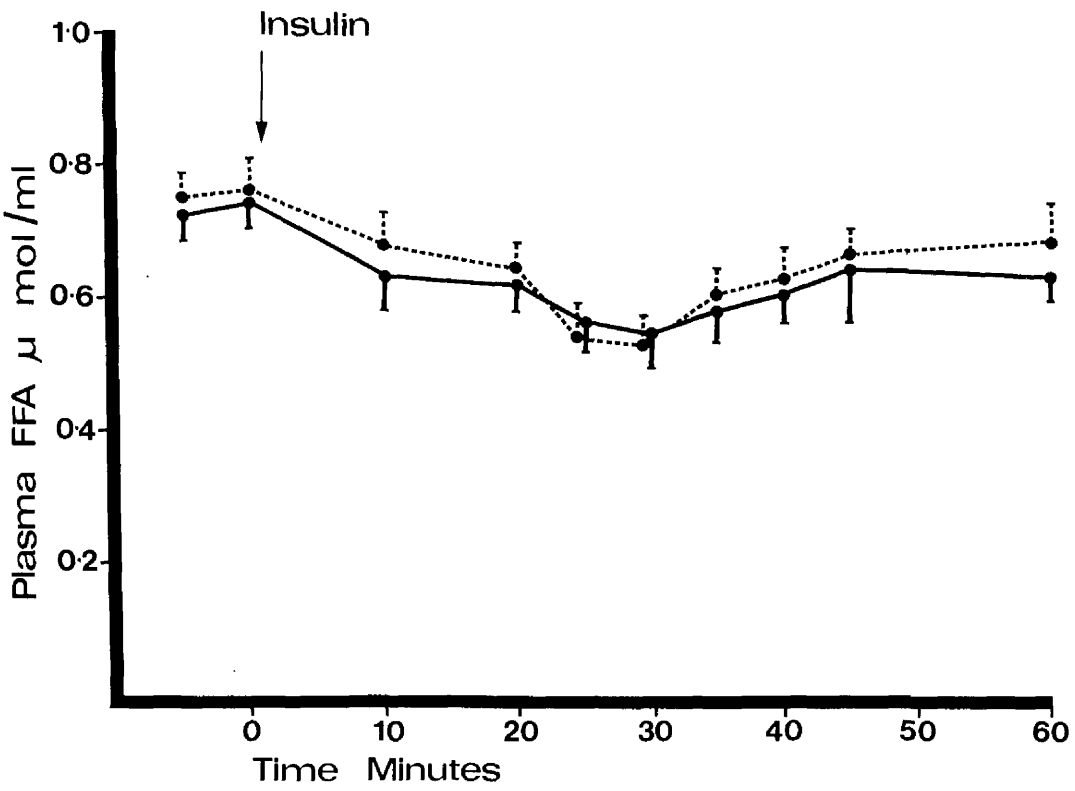
TABLE 5.III Growth hormone concentrations in mu/l before and after the injection of insulin (0.1 U/kg body weight) in 7 alcoholics and the mean concentrations in 10 control subjects

	Time (min)									
Patients	0	0	10	20	25	30	35	40	45	60
R.A.	0.2	0.2	0.2	0.2	0.6	12.6	29.6	50.2	69.4	71.0
T.B.	1.8	1.0	0.8	3.0	20.4	36.4	46.8	43.8	72.6	53.8
T.C.	0.4	0.4	0.4	0.6	1.4	5.2	26.4	31.8	44.8	27.8
J.L.	0.2	0.4	0.2	0.2	0.4	0.4	4.2	12.2	21.2	19.2
A.S.	2.8	2.0	2.6	3.2	3.0	3.6	5.0	6.8	4.8	3.6
J.S.	0.4	0.2	0.2	0.4	0.2	0.8	0.8	0.6	1.6	1.4
J.D.	2.6	1.6	1.2	1.0	1.0	1.0	1.0	1.0	0.4	0.6
Mean	1.2	0.8	0.8	1.2	3.9	8.6	16.3	21.0	30.7	25.3
S.E.M.	0.4	0.4	0.4	0.6	2.8	5.0	6.8	7.8	11.8	10.4
Controls										
Mean	7.8	7.8	7.8	8.0	8.0	11.0	29.4	49.4	71.4	78.6
S.E.M.	1.6	1.6	1.6	1.0	4.0	3.6	8.4	25.8	17.0	9.0
Significance of difference	P<0.001	P<0.001	P<0.001	P<0.001	NS	NS	NS	NS	P<0.02	P<0.001

TABLE 5.IV Plasma corticosteroids (mmol/l) before and after the injection of insulin (0.1 U/kg body weight) in 7 alcoholics and the mean concentrations in 6 control subjects.

	Time (min)									
Patients	0	0	10	20	25	30	35	40	45	60
R.A.	445	445	632	444	439	508	569	461	519	439
T.B.	237	293	351	433	395	444	450	326	124	226
T.L.	502	414	535	502	406	552	573	828	1041	894
J.L.	444	345	287	254	340	238	276	425	693	707
A.S.	472	461	406	406	400	395	607	627	574	579
J.S.	287	315	130	110	124	157	179	163	179	151
J.D.	439	422	389	342	389	406	317	243	163	179
Mean	404	385	390	356	356	386	439	439	523	446
S.E.M.	38	25	62	51	40	54	71	87	123	107
Controls										
Mean	453	475	505	577	533	505	624	820	894	767
S.E.M.	58	55	141	149	105	80	94	102	102	102
Significance of difference	NS	NS	NS	NS	NS	NS	NS	P<0.01	P<0.05	P<0.05

FIG 5.I Plasma free fatty acid concentrations (μmol/ml, mean ± SEM) in seven alcoholics (⊙——●) and in ten control subjects (⊙----●) before and after the injection of insulin (0.1U/ kg body weight).


Insulin
1·0
0·8
0·6
0·4
0·2
Plasma FFA μ mol/ml
0
10
20
30
40
50
60
Time Minutes

DISCUSSION

The results presented in this chapter suggest that there is a depression of hGH release in response to insulin induced hypoglycaemia in alcoholic withdrawal. Hypoglycaemia (Roth et al. 1963), stress (Schalch, 1967), diurnal variation (Glick & Goldsmith, 1968) and a fall in plasma FFA (Irie et al. 1967) have all been reported as possible stimuli producing an increase in serum hGH . Increasing age may be associated with a lowering of hGH responses (Maany et al. 1975). It is possible that some of these factors operated in the control subjects tending to augment their hGH responses. However, the concentrations of blood glucose and plasma FFA were similar in both groups, and all the patients and subjects developed adequate hypoglycaemia (blood glucose < 2.2 mmol/l) during the investigation. The studies were carried out at the same time in the morning and both groups were matched for age so that this would not be an important factor. In addition, the hGH concentrations in the control subjects are very similar to those reported in normal subjects given the same dose of insulin (Roth et al., 1963). A deficient growth hormone response to insulin hypoglycaemia similar to that found in some alcoholics in the present study has

been reported in a single alcoholic in whom hGH responsiveness returned to normal after 9 months abstinence from alcohol (Adreani, Tamburrano and Javicoli, 1976). Wright, Fry, Merry and Marks (1976) reported that they could not find any consistent abnormality in the growth hormone response to insulin hypoglycaemia in alcoholics but the patients they studied were drinking up to the time of the investigation whereas the patients in the present study had been abstinent for a few days. This suggests that the impaired growth hormone responses may be a feature of alcohol withdrawal.

The results for the cortisol response are similar to those of Merry & Marks (1972), who also found that the normal increase in cortisol in response to insulin hypoglycaemia is impaired in a number of alcoholics. Two patients who shared a fall in cortisol concentrations had no hGH response, suggesting that these altered responses may be related to a general impairment of release of pituitary hormones.

The depression of hypothalamic/pituitary function reported here may be related to withdrawal following the continued and prolonged stimulation of the hypothalamus

by alcohol. Recent evidence (Lal, Tolis, Martin, Brown and Guyda, 1975) suggests that there are dual noradrenergic/dopaminergic hGH modulating mechanisms. Stimulation of α-adrenergic receptors (Blackard, 1970) and dopaminergic receptors (Lal et al., 1973) increases hGH concentrations, whereas β-receptor stimulation inhibits hGH release (Massara & Camanni, 1971). In particular, the hGH response to insulin hypoglycaemia appears to be mediated by α-receptors as the response is blocked by phentolamine (Blackard & Heidingsfelder, 1968). It is possible that the impaired hGH release in some of the alcoholics in the present investigation is due to an alteration of catecholaminergic systems modulating hGH release. Further investigation of a larger group of patients is required to confirm the present findings and to determine if prolonged alcohol consumption and alcohol withdrawal alter mechanisms modulating hGH and ACTH, and to assess the extent to which the release of other pituitary hormones is required.

SUMMARY

1. The growth hormone response to insulin induced hypoglycaemia was studied in 7 alcoholic in-patients who had been abstinent for 2-11 days and in 10 normal controls. Blood samples were taken at intervals after the injection of soluble insulin (0.1 U/kg body weight).

2. The growth hormone response was impaired in 4 of the alcoholics and the depression was not related to differences in blood glucose or plasma-free fatty acids. The cortisol response was also impaired in 5 patients.

3. The results suggest that a number of alcoholics observed after alcohol withdrawal may have a depression of hypothalamic/pituitary function.

CHAPTER 6

GROWTH HORMONE, PROLACTIN AND CORTISOL RESPONSE TO INSULIN HYPOGLYCAEMIA IN ALCOHOLICS

INTRODUCTION

Chronic alcohol consumption has a number of important interactions with the endocrine system (Gordon and Southern, 1977). In particular, a proportion of alcoholics have an impaired cortisol response to oral alcohol and hypoglycaemia (Merry and Marks, 1972). The results presented in the previous chapter demonstrate that a number of alcoholics investigated shortly after cessation of drinking have impaired growth hormone (hGH) responses to hypoglycaemia. It was decided to extend these studies by investigating growth hormone, prolactin (hPRL) and corticosteroid responses and relevant features of the patient's clinical history was also examined.

METHODS

Patients and subjects

Twenty-four male patients, mean age 45 years (range 25-65 years) with a diagnosis of a progressive alcohol problem were investigated 2-7 days after they had stopped drinking. The patients had all given their informed consent to the study. Only patients who gave a history of symptoms of physical dependence on alcohol were studied and patients known to have hepatic cirrhosis and hypokalameic or jaundiced patients were excluded. Liver function tests including γ-glutamyl

transpeptidase were routinely performed on admission and suggested that the patients had been drinking before admission but did not demonstrate marked liver damage. Growth hormone and prolactin responses were also studied in ten normal healthy controls (5 male, 5 female) mean age 42 years (range 32-51 years). In addition, the cortisol response was studied in 6 male control subjects, mean age 38 years (range 23-51 years).

Procedure

Assessment of the clinical features of alcoholism was performed independently using a standard questionnaire which was given when the patient no longer displayed signs or symptoms of alcohol withdrawal. The information was corroborated by a relative when possible. Severity of withdrawal symptoms was estimated on the basis of an objective rating scale:-

Score or Group	Symptoms
0	No symptoms.
1	Tremor, insomnia, autonomic nervous system manifestations.
2	Delirium tremens.

Blood samples were withdrawn from a cannula in an antecubital vein before and at 10, 20, 25, 30, 35, 40, 45 and 60 minutes after the injection of soluble insulin (0.1 U/kg body weight) via the cannula. Blood samples were analysed for glucose and plasma samples were analysed for hGH, hPRL and cortisol.

RESULTS

The clinical history of the patients (Table 6.I) demonstrated that all the patients had consumed relatively large amounts of alcohol on a regular basis for a number of years. All the patients had a history of an alcohol problem of not less than 2 years and some of the patients had been admitted to hospital for treatment of an alcohol problem on a number of previous occasions.

Blood glucose (Table 6.II)

Basal blood glucose concentrations were similar in both patients and controls. The minimum concentration after the injection of insulin demonstrated that all the patients developed adequate hypoglycaemia (blood glucose < 2.2. mmol/l).

Plasma growth hormone (Table 6.II)

Basal hGH concentration in the patients were generally lower than the controls. Nine of the twenty four patients studied had an inadequate hGH response with a peak concentration less than 10 mu/l.

Plasma prolactin (Table 6.III)

Basal hPRL concentrations were similar in both patients and controls. Nine of the patients demonstrated a fall or no change from basal hPRL concentrations whereas the remaining 15 patients had at least a small rise in hPRL concentrations. Of the nine patients who showed a fall or no change in hPRL concentrations, eight had impaired hGH responses.

TABLE 6.I Features of clinical history in 24 alcoholics

Patient No.	Age Years	Previous admissions	Duration of drinking years	Duration of alcohol problem years	Admitted alcohol intake g/alcohol/week	Withdrawal symptoms Score
1	33	10	10	5	1260	0
2	34	4	15	15	1568	1
3	50	2	30	6	1470	1
4	44	1	25	2	3360	2
5	52	0	30	5	1680	0
6	38	0	20	15	2499	1
7	42	0	20	2	2457	0
8	42	0	15	3	1680	1
9	53	10	30	13	3276	0
10	41	1	20	12	1313	0
11	39	5	20	7	3665	1
12	46	2	30	22	3266	2
13	41	3	25	14	1638	1
14	48	0	30	10	1680	0
15	30	6	12	3	1736	0
16	52	1	30	20	2457	2
17	25	0	8	3	1229	0
18	48	1	20	8	2205	1
19	65	0	40	20	1680	2
20	47	0	30	7	2310	1
21	50	6	30	15	1628	0
22	50	8	20	5	3360	1
23	49	2	30	4	1680	1
24	53	0	35	10	-	2

TABLE 6.II Basal concentrations of blood glucose (mmol/1), and plasma hGH (mu/1) and minimum concentration of glucose and concentration of hGH at 45 and 60 minutes after the injection of insulin in 24 alcoholics.

Patient	Blood glucose mmol/1		Plasma hGH mu/1		
No.	rest	minimum	rest	45 min	60 min
1	4.68	1.47	2.6	60.4	25.2
2	5.4	1.4	4.2	88.6	89.0
3	5.8	2.0	2.0	55.0	41.6
4	5.0	0.8	2.8	4.8	3.6
5	4.4	1.8	0.2	69.4	71.0
6	4.6	1.0	8.2	33.2	65.4
7	5.6	0.8	0.5	21.0	19.9
8	4.8	1.4	2.5	0.4	0.6
9	5.4	1.0	4.2	95.8	81.4
10	4.2	1.2	0.2	21.2	19.1
11	4.2	1.1	1.8	72.6	53.8
12	6.3	1.6	0.2	0.2	0.2
13	5.9	1.4	0.4	31.8	44.8
14	6.0	1.8	2.1	3.6	5.8
15	5.8	1.3	0.4	89.2	135.2
16	4.5	1.0	0.8	3.0	2.4
17	6.5	1.4	0.2	0.2	0.4
18	6.1	1.1	0.7	12.7	24.0
19	6.8	1.2	2.8	5.3	1.8
20	5.0	1.5	0.4	1.6	1.4
21	4.7	1.1	2.4	15.6	27.0
22	4.5	1.7	4.8	20.8	39.0
23	4.5	1.0	0.8	63.2	89.4
24	5.9	1.6	0.6	1.2	8.0
Controls					
Mean	4.2	1.2	7.8	71.4	78.6
SEM ±	0.3	0.2	1.6	17.0	9.0

TABLE 6.III Basal concentrations of hPRL (mu/1) and cortisol (mmol/1) and concentrations at 45 and 60 minutes after the injection of insulin in 24 alcoholics.

Patient	Plasma hPRL mu/1			Plasma cortisol mmol/1		
No.	rest	45 min	60 min	rest	45 min	60 min
1	120	600	360	489	908	756
2	300	1580	1620	345	566	649
3	160	120	120	541	1065	1228
4	180	160	160	472	574	519
5	120	600	400	613	519	439
6	160	360	300	445	745	552
7	160	700	580	414	657	668
8	60	30	20	439	163	179
9	360	650	900	345	317	436
10	160	240	200	444	693	707
11	180	1640	1740	235	400	502
12	160	120	100	800	259	287
13	120	140	140	502	1041	894
14	120	120	80	469	914	527
15	200	200	240	474	500	500
16	120	100	90	469	524	745
17	240	140	140	497	497	392
18	100	160	240	524	635	607
19	360	360	340	381	351	323
20	860	720	800	287	179	157
21	240	850	850	337	585	773
22	280	400	460	442	657	668
23	100	420	620	226	580	276
24	240	180	100	745	489	444
Controls						
Mean	207	520	530	453	894	767
SEM ±	24	70	81	58	102	102

Plasma cortisol (Table 6.III)

Thirteen patients and all the controls showed an increase above basal cortisol concentrations greater than 200 mmol/l. In 4 patients plasma cortisol increased, but the rise was less than 200 mmol/l and in the 7 remaining patients, plasma cortisol decreased from basal levels. Six of the patients who showed a decrease from basal cortisol concentrations had impaired hGH responses.

Relationship between hGH, hPRL and cortisol responses (Table 6.IV)

In an attempt to examine if there was an impairment of all three hormone responses in some patients, the increase or decrease from basal concentration of hGH at 45 and 60 minutes was correlated with the corresponding change in hPRL and cortisol concentration. The only significant correlation found was between the hGH and hPRL responses at 45 and 60 minutes.

Relationship between hGH response and features of clinical history

The increase or decrease above basal concentrations of hGH at 45 and 60 minutes was correlated with age, duration of drinking, duration of alcohol problem and admitted alcohol intake. In no case was a significant correlation found.

The concentrations of the various hormones at 45 and 60 minutes were grouped according to the patient's score for withdrawal symptoms.

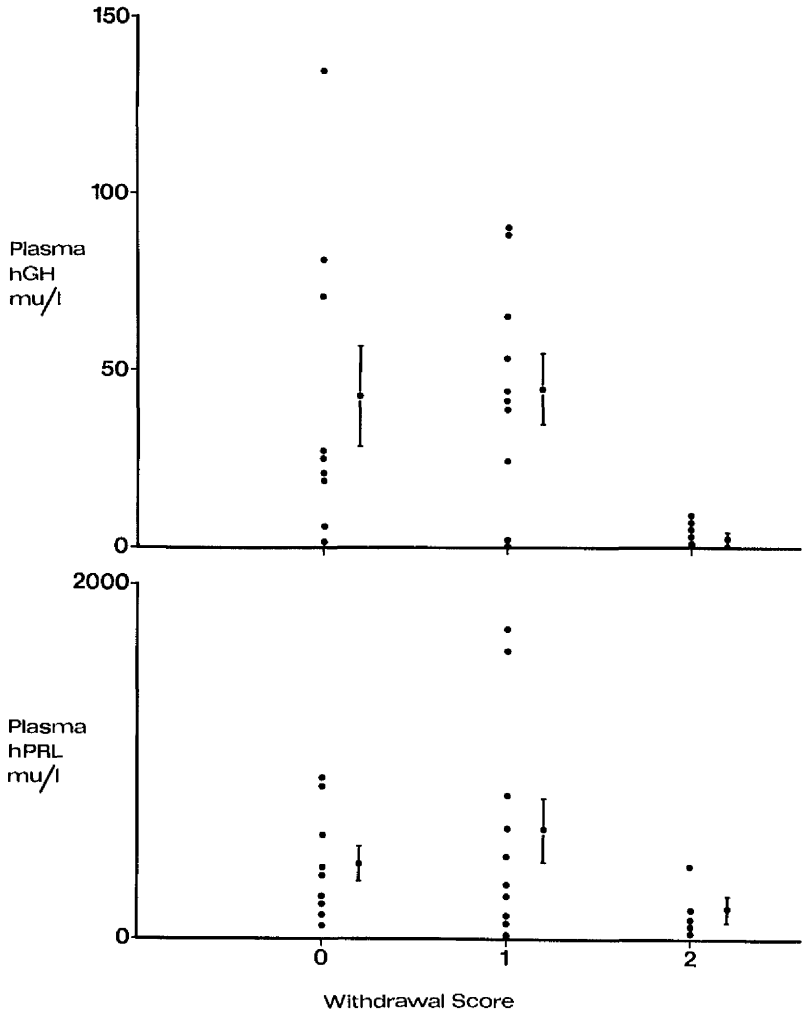
Growth hormone concentrations in patients with a withdrawal score of 2 were significantly lower than those with scores of 0 or 1 at 45 minutes ($P < 0.014$ and $P < 0.008$ respectively) and at 60 minutes ($P < 0.024$ and $P < 0.002$ respectively). (Fig. 6.I). There was no significant difference between the hGH concentration of Groups 0 and 1. Prolactin concentration of withdrawal score group 2 were lower than for group 0 and 1 at 45 and 60 minutes but the difference was not significant at the 5% level. There was no significant difference between plasma cortisol concentrations for any of the withdrawal score groups at either 45 or 60 minutes.

TABLE 6.IV

Relationship between the increase or decrease from basal concentrations of hGH, hPRL and cortisol at 45 and 60 minutes after the injection of insulin (0.1 U/kg body weight) in 24 alcoholics.

Time	Increase or decrease from basal concentrations		Correlation coefficient	Significance
Mins.	Y.	X.		
45	hGH	hPRL	0.571	$P < 0.005$
	hGH	Cortisol	0.279	N.S.
60	hGH	hPRL	0.447	$P < 0.01$
	hGH	Cortisol	0.203	N.S.

FIG 6.I Increase or decrease from basal concentrations (mu/l) of growth hormone (hGH) and prolactin (HPRL) 60 minutes after the injection of insulin (0.1 U/kg body weight) in 24 alcoholics with differing severity of withdrawal symptoms (see text).


150
100
50
0
Plasma
hGH
mu/l
2000
0
Plasma
hPRL
mu/l
0
1
2
Withdrawal Score

DISCUSSION

The results presented here confirm the results of earlier studies which demonstrate that hGH responses are impaired in some alcoholics (Andreani, Tamburrano and Javicoli, 1976; Chapter 5). All the patients developed adequate hypoglycaemia for the hGH response (blood glucose $<$ 2.2 mmol/l) and nine of the patients had impaired hGH responses. Prolactin concentration either decreased or showed a minimal increase in eight of the patients with impaired hGH responses. Seven patients showed a decrease from basal cortisol concentrations and six of them had an impaired hGH response. The present observation that a proportion of alcoholics have an impaired cortisol response to hypoglycaemia is consistent with other published findings (Merry and Marks, 1972; Chapter 5). The presence of an impairment in the response of all three hormones in some patients suggests that there may be a general impairment of release of these hormones in some patients.

Impairment of release of all three hormones might be indicated by a significant positive correlation between the three responses in the patients. Significant correlations were found between the hPRL and hGH responses at 45 and 60 minutes. However, hPRL responses were relatively small and variable and a greater degree of hypoglycaemia may be required for a more consistent hPRL response (Noel, Suh and Frantz, 1971). Although there was no significant correlation between the hGH and cortisol responses, it is possible that individual

differences in adrenal responsiveness or timing of ACTH release may exist. The results suggest that both hGH and hPRL responses were impaired in some patients but further investigation of ACTH responses will be required to determine if the responses of all three hormones are impaired.

The most consistent abnormality found in the present study was the presence of an impaired hGH response . No significant correlation was found between the hGH response and any of the recorded features of the clinical history. Growth hormone responses are known to be related to age (Maany, Frazer and Mendels, 1975), but it is unlikely that this relationship would be apparent in the present study since age differences were relatively small and some of the patients demonstrated impaired responses. If the impaired hGH response was related to chronic alcohol consumption, then factors like duration of drinking problem and admitted alcohol intake might have shown a significant relationship, but there was no evidence of this in the present investigation.

The hGH responses in the five patients with the most severe withdrawal symptoms were impaired suggesting that alcohol withdrawal may be required to demonstrate this effect. Wright, Merry, Fry and Marks (1976) investigated hypothalamic/pituitary function in alcoholics and reported that the hGH response to

insulin hypoglycaemia was normal. However, their patients were drinking up to the time of investigation and had not experienced alcohol withdrawal. An impaired hGH response to insulin hypoglycaemia has been reported in a single abstinent alcoholic but normal responsiveness had returned when the patient was subsequently reinvestigated (Andreani et al., 1976). It appears that abstinence or withdrawal of alcohol from the alcoholic may be required to demonstrate an impaired hGH response.

The presence of withdrawal symptoms is diagnostic of alcohol dependence but the mechanisms involved in the development of alcohol withdrawal syndrome are not fully understood (Hore, 1977). The significance of the present observation of impaired stress responses in some recently abstinent alcoholics requires further investigation as it may have important implications for the management of alcohol withdrawal syndrome.

SUMMARY

1. Growth hormone (hGH), Prolactin (hPRL) and cortisol responses to insulin induced hypoglycaemia were studied in 24 patients with a diagnosis of a progressive alcohol problem who had been abstinent for 2-7 days. Blood samples were taken at intervals after the injection of soluble insulin (0.1 U/kg body weight). All the patients developed adequate hypoglycaemia (blood glucose < 2.2 mmol/l) and nine of the patients had impaired hGH responses.

2. Prolactin concentrations showed a fall or no change from basal levels in nine patients and eight of them also had impaired hGH responses. In seven patients cortisol concentrations decreased from basal levels and six of these patients had imapired hGH responses. A number of patients had impairment of all three hormone responses and significant correlations were found between the hGH and hPRL responses at 45 and 60 minutes.

3. There was no correlation between the hGH response and age, duration of drinking, duration of alcohol problem or admitted alcohol intake. Growth hormone responses were significantly lower in the patients who had experienced the severest withdrawal symptoms. The observations of impaired stress responses in some

recently abstinent alcoholics may have important implications for the management of alcohol withdrawal syndrome.

CHAPTER 7

THE GROWTH HORMONE AND PROLACTIN RESPONSES TO BROMOCRIPTINE IN NORMAL SUBJECTS AND IN ACROMEGALY

INTRODUCTION

Release of hGH and hPRL is known to be modulated by catecholamines and serotonin (Imura et al., 1973; Nakai et al., 1973; Fronham and Stachura, 1975; L a l et al., 1975). Evidence for the involvement of dopamine in the regulation of hGH and hPRL release includes the observations that bromocriptine, a specific dopamine receptor agonist, stimulates hGH and suppresses hPRL release in normal subjects (Cammani et al., 1975; Tolis et al., 1975) and suppresses both hGH and hPRL in acromegaly (Cammani et al., 1975; Thorner, Chait, Aitken, Benker, Bloom, Mortimer, Sanders, Stuart Mason and Besser, 1975). The action of bromocriptine appears to be specific to dopamine receptors and it may therefore be of value in investigating dopaminergic regulation of hGH and hPRL release in patients with evidence of altered hypothalamic function. Before carrying out such studies it was necessary to confirm these responses to bromocriptine and to ensure that the responses were not related to hypoglycaemia or a fall in FFA concentrations.

Acromegaly is a disorder involving abnormal secretion of hGH usually associated with an anterior pituitary tumour and is characterised by marked elevation of basal hGH concentrations. An opportunity arose to investigate the effect of bromocriptine in a patient with this disorder and to monitor hGH and hPRL

concentrations during long term treatment with this drug. The results of these studies in normal subjects and in acromegaly are presented in this chapter.

I RESPONSE TO BROMOCRIPTINE IN NORMAL SUBJECTS

METHODS

Seven normal subjects (4 male, 3 female) aged 23-55 years (mean 44) were studied. The investigations were carried out in the morning after an overnight fast and the subjects remained at rest, lying down, throughout the investigation. Blood samples were taken from a cannula in an antecubital vein at 30, 60, 90, 120, 130, 140, 150, 160, 170, 180 and 210 minutes after oral administration of bromocriptine (2.5 mg). Blood samples were analysed for glucose and glycerol and plasma samples analysed for FFA, hGH and hPRL. Significance of difference was examined using the Mann-Whitney non-parametric U-test for small samples (Mann and Whitney, 1947).

RESULTS

Plasma hGH (Fig 7.I)

Concentrations of hGH increased 2 hours after bromocriptine and were significantly greater ($P < 0.05$, 0.05, 0.02 and 0.05) than basal concentrations at 150, 160, 170 and 180 minutes.

Plasma hPRL (Fig 7.I)

Prolactin concentrations decreased after bromocriptine, but although the results suggest suppression of hPRL, concentrations after bromocriptine were not significantly lower than basal concentrations.

Plasma FFA and blood glycerol (Fig 7.II)

There was no significant change from basal concentrations of FFA or blood glycerol at any time during the investigation.

Blood glucose

Blood glucose concentrations after bromocriptine were not significantly different from basal concentrations.

II. RESPONSE TO BROMOCRIPTINE IN ACROMEGALY

METHOD

Patient

One male patient with a diagnosis of acromegaly was studied. The patient had received radiotherapy treatment four years previously. Symptoms had persisted and one year later he underwent cryosurgery for the destruction of the anterior pituitary tumour, but basal hGH concentrations have continued to be elevated for the last three years following this procedure.

Procedure

Investigations involved giving bromocriptine orally and collecting blood samples at appropriate intervals of time from a cannula in an antecubital vein. Growth hormone and hPRL

responses to 2.5 and 10 mg of bromocriptine were studied on two separate occasions. Hormone levels were monitored during long term treatment by taking blood samples by venepuncture when the patient attended the hospital. Plasma samples were analysed for hGH and hPRL.

RESULTS

Plasma hGH and hPRL (Fig 7.III)

Basal hGH and hPRL concentrations were elevated on both occasions before treatment. Administration of 2.5 mg and 10.0 mg of bromocriptine on separate occasions was associated with a fall in hGH and hPRL concentrations. After treatment with 2.5 mg/day for ten days, hGH and hPRL levels were found to be only slightly lower than pre-treatment levels (Fig 7.IV). The dose of bromocriptine was gradually increased until the patient was receiving 10 mg/day and treatment at this dosage was associated with suppression of hGH and hPRL concentrations. At one stage during treatment the patient exhausted his supply of the drug and hGH and hPRL levels returned to pre-treatment levels but were again suppressed when treatment was restarted.

FIG 7.I Plasma hGH and hPRL concentrations (mu/l, mean $\pm$ S.E.M.) in seven normal subjects before and after oral administration of bromocriptine (2.5 mg).

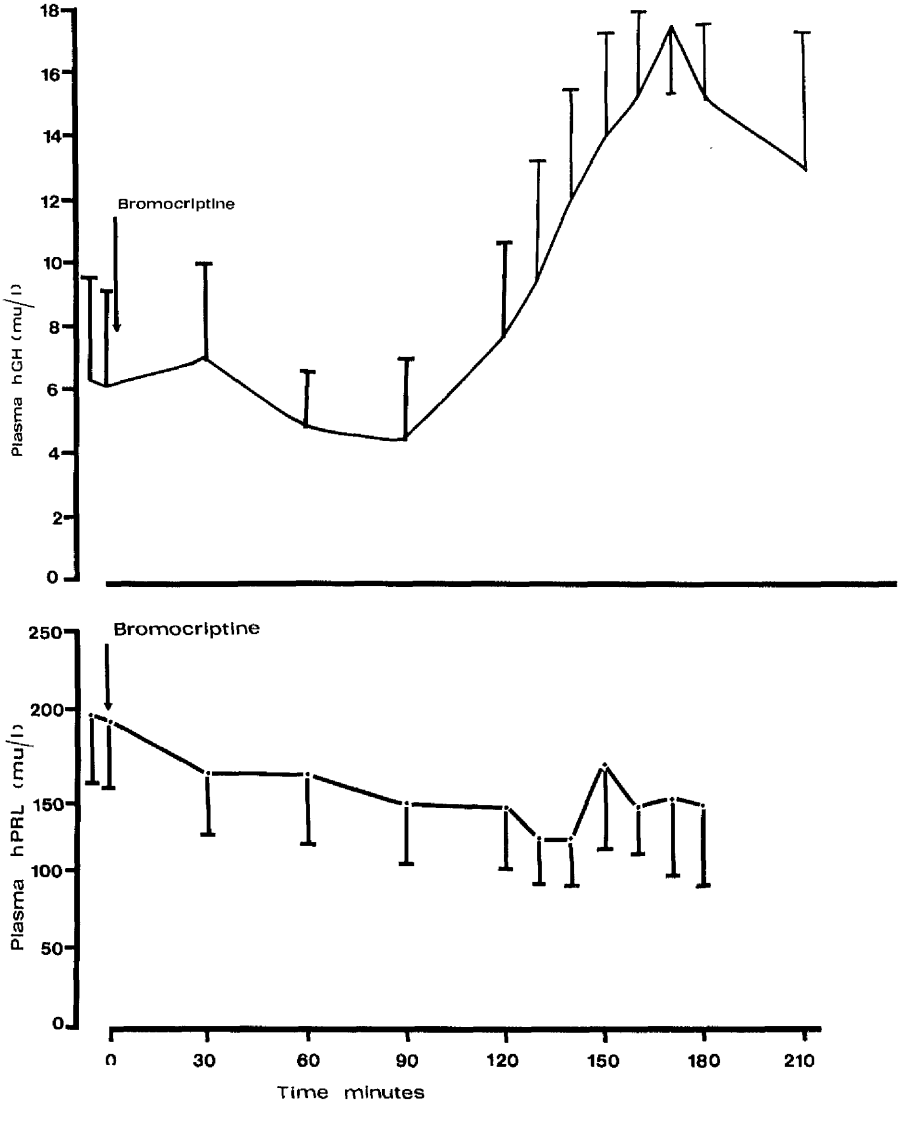

Bromocriptine
Plasma hGH (mu/l)
18
16
14
12
10
8
6
4
2
0
Bromocriptine
Plasma hPRL (mu/l)
250
200
150
100
50
0
0
30
60
90
120
150
180
210
Time minutes

FIG. 7.II Plasma FFA and blood glycerol concentrations (μmol/ml, mean ± S.E.M.) in seven normal subjects before and after oral administration of bromocriptine (2.5 mg).

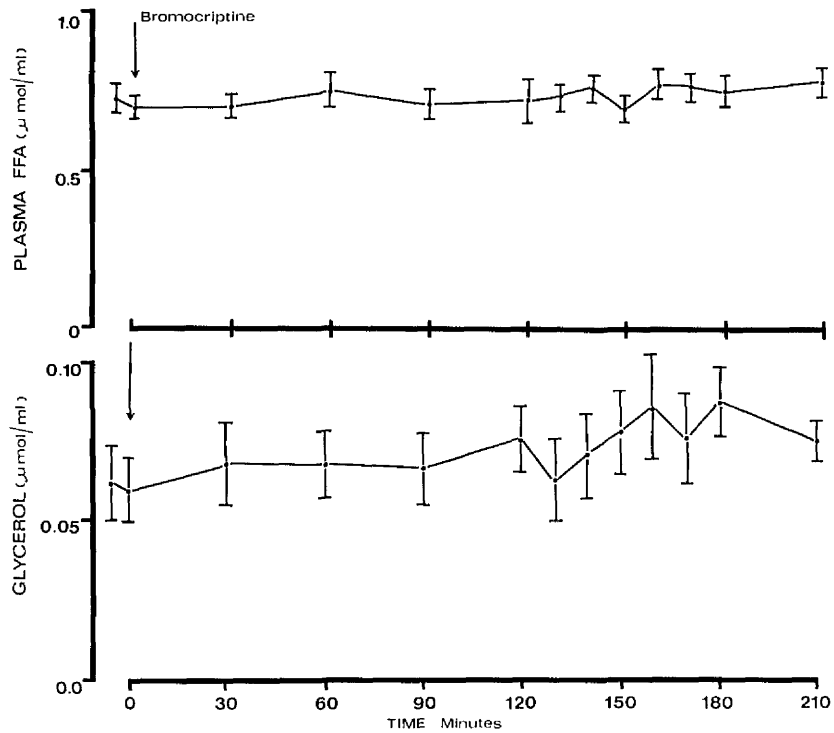

Bromocriptine
PLASMA FFA (μ mol/ml)
1.0
0.5
0
GLYCEROL (μ mol/ml)
0.10
0.05
0.0
0
30
60
90
120
150
180
210
TIME Minutes

FIG. 7.III Plasma hGH and hPRL concentrations (mu/l) before and after administration of bromocriptine in a patient with acromegaly.

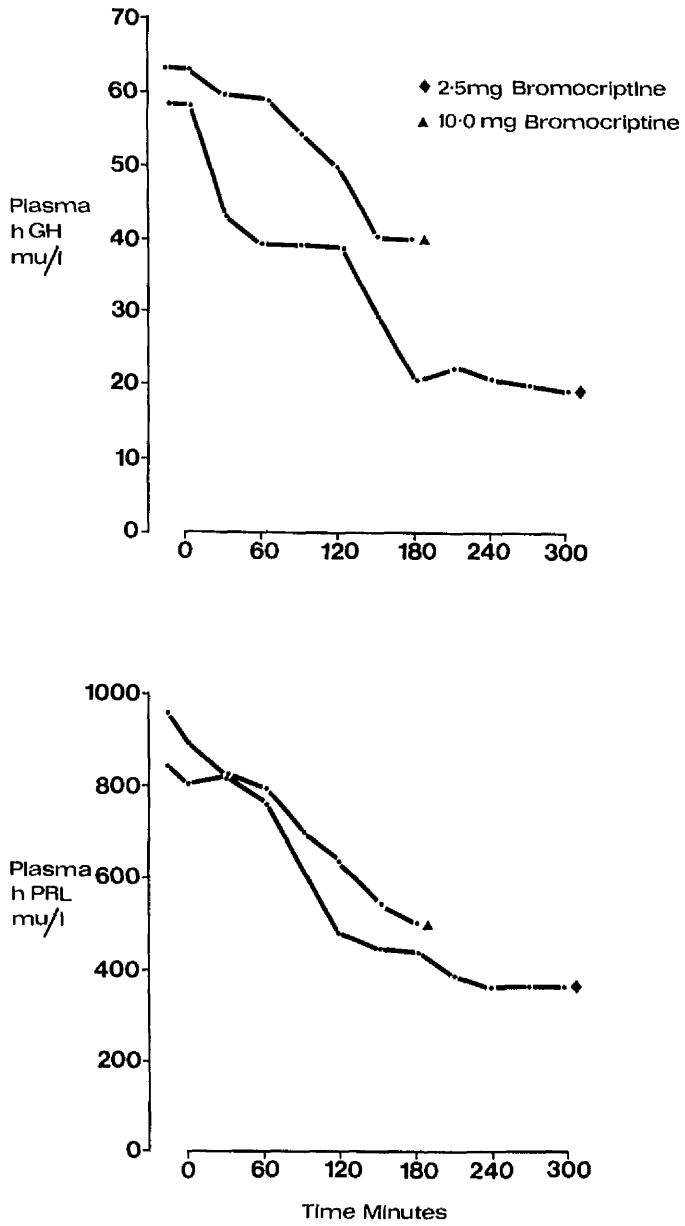
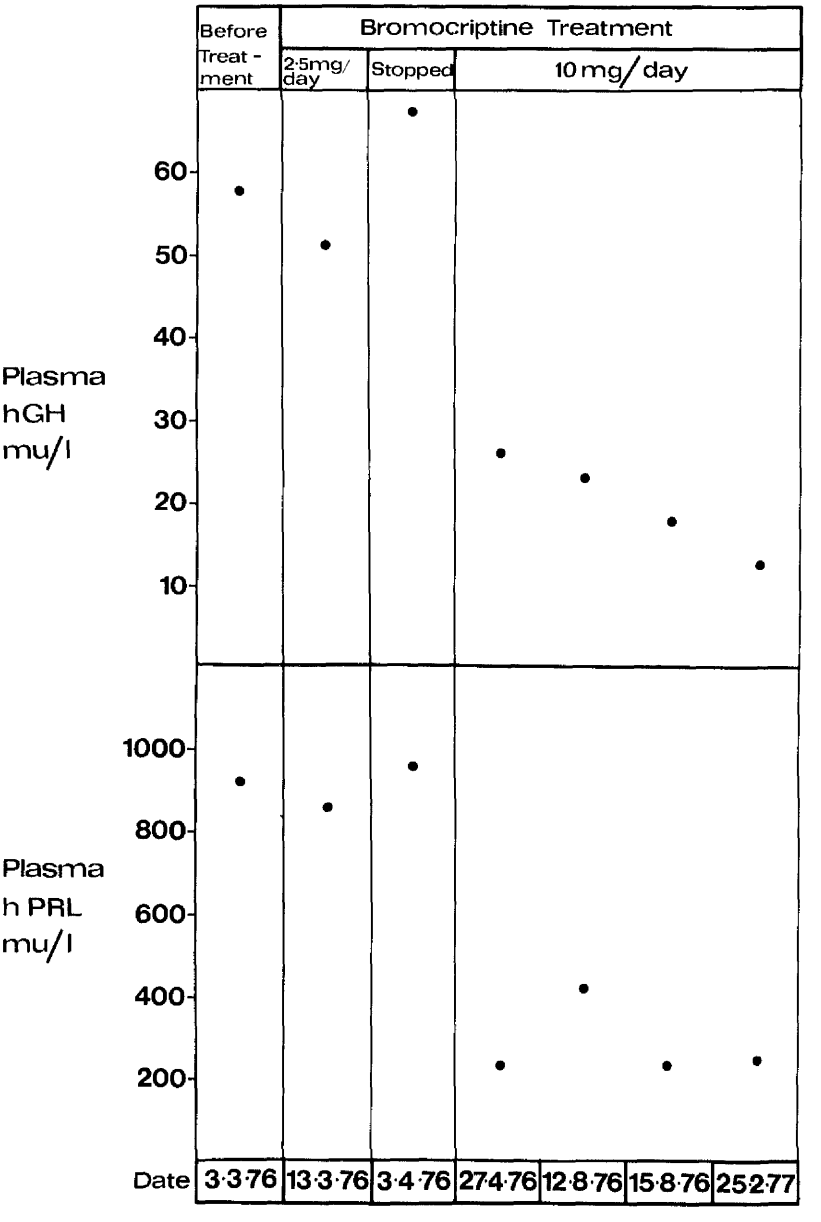

70
60
50
40
30
20
10
0
Plasma h GH mu/l
♦ 2·5mg Bromocriptine
▲ 10·0 mg Bromocriptine
0
60
120
180
240
300
1000
800
600
400
200
0
Plasma h PRL mu/l
0
60
120
180
240
300
Time Minutes

FIG. 7.IV Plasma hGH and hPRL concentrations (mu/l) before and during treatment with bromocriptine in a patient with acromegaly.


Before Treat - ment
Bromocriptine Treatment
2·5mg/ day
Stopped
10 mg/ day
Plasma hGH mu/l
60
50
40
30
20
10
Plasma h PRL mu/l
1000
800
600
400
200
Date
3·3·76
13·3·76
3·4·76
27·4·76
12·8·76
15·8·76
25·2·77

DISCUSSION

The results presented in this chapter demonstrate that bromocriptine produces significant elevation of hGH in normal subjects and suppresses hGH and hPRL in acromegaly. These results are in agreement with other published findings for normal subjects (Del Pozo et al., 1972; Tolis et al., 1975) and for a number of acromegalics (Cammani et al., 1975; Thorner et al., 1975).

Growth hormone release is known to follow a fall in FFA concentrations (Hartog et al., 1967) while hGH and hPRL release follows a fall in blood glucose (Hunter et al., 1965a; Noel et al., 1971). There was no evidence for a significant change in blood glucose concentrations after bromocriptine in the present investigation. Tolis and co-workers (1975) reported a rise in plasma FFA 4-5 hours after oral administration of bromocriptine but there was no significant alteration of plasma FFA or blood glycerol concentrations during the $3\frac{1}{2}$ hours after bromocriptine administration in the present study.

In the absence of a change in known metabolic stimuli the present results suggest that bromocriptine acts on hypothalamic or pituitary receptors regulating hGH and hPRL release. Dopamine is known to be present in relatively large quantities in the median eminence and the close relationship between dopamine containing granules to capilliaries of the portal plexus suggests that dopamine may have a role in the regulation of hGH release

(Fuxe and Hokfelt, 1969). This suggestion is supported by the observations that L-dopa the precursor of dopamine and the dopamine receptor agonist apomorphine both elevate hGH in normal subjects (Boyd et al., 1970; Maany et al., 1975). Both L-dopa and apomorphine are thought to act on CNS loci (Muller, 1973). The present observation of hGH release in response to bromocriptine probably involves stimulation of hypothalamic dopamine receptors regulating GRF or GRIF.

Prolactin release is known to be under inhibitory control from the hypothalamus and there is evidence that dopamine has prolactin release inhibiting properties (Kamberi, 1973; Zacur et al., 1976). Further evidence for the involvement of dopamine in the regulation of hPRL release comes from the observations that phenothiazines, which are known to block dopamine and noradrenaline receptors in the CNS (Sourkes, 1975), produce elevation of hPRL levels (Apostolakis et al., 1972; Frantz et al., 1972; Beumont et al., 1974). In the present study there was evidence of suppression of hPRL by bromocriptine in normal subjects but because of variation in hPRL levels this suppression was not statistically significant. However, the marked suppression of hPRL in the acromegalic patient does suggest that bromocriptine inhibits hPRL release.

The acromegalic patient had elevated basal concentrations of both hGH and hPRL and this is consistent with reports of the

elevation of both hormones in a number of acromegalics (Franks, Jacobs and Nabarro, 1976). Long term treatment with bromocriptine suppressed hGH and hPRL levels in the patient and this finding is in agreement with the results of a number of trials of bromocriptine in the treatment of acromegaly (Thorner et al., 1975; Chiodini, Liuzzi, Botalla, Oppizzi, Muller and Silvestrini, 1975; Sachdev, Gomez-Pan, Tunbridge, Duns, Weightman and Hall, 1975). Bromocriptine is known to inhibit release of hGH and hPRL from anterior pituitary tumour cells 'in vitro' and this suggests that the suppression of hGH and hPRL in acromegaly may involve stimulation of dopamine receptors on pituitary cells (Mashitev, Adams and Holley, 1977). In the present investigation the suppression of hPRL in normal subjects and in the acromegalic patient occurred shortly after bromocriptine administration suggesting a direct action on the pituitary whereas the rise in hGH in normal subjects was delayed. The possibility that the hGH response in normal subjects involves hypothalamic stimulation whereas suppression of hPRL involves pituitary receptors requires further investigation.

The results presented in this chapter confirm the results of other research workers and demonstrate that bromocriptine produces an elevation of hGH in normal subjects. Bromocriptine produced suppression of both hGH and hPRL in an acromegalic patient and these results suggest that bromocriptine may be of value in the treatment of acromegaly.

SUMMARY

1. Growth hormone and hPRL responses to oral bromocriptine were investigated in seven normal healthy control subjects and in one patient with acromegaly.

2. Bromocriptine administration in normal subjects produced a significant increase in hGH concentrations and the hGH response was not related to a fall in blood glucose or plasma FFA concentrations. Prolactin concentrations in the normal subjects were not significantly different after bromocriptine administration.

3. Administration of 2.5 mg and 10 mg of bromocriptine on two separate occasions produced suppression of both hGH and hPRL concentrations in a patient with acromegaly. After treatment with 2.5 mg/day for 10 days hGH and hPRL concentrations were only slightly lower than pre-treatment levels but treatment with 10 mg/day was associated with marked suppression of both hGH and hPRL concentrations in the acromegalic.

4. The results confirm the findings of other investigators and provide further evidence for the involvement of dopamine in the regulation of hGH and hPRL release. The marked suppression of hGH and hPRL during bromocriptine treatment in acromegaly suggests that bromocriptine may be of value in the treatment of this disorder.

CHAPTER 8

THE EFFECT OF FLUPHENAZINE ON BASAL PROLACTIN CONCENTRATIONS

INTRODUCTION

Treatment with phenothiazine derivatives is known to be associated in some patients with symptoms of galactorrhoea (Gade and Heinrich, 1955) and menstrual disorders (Polishuk and Kulcsar, 1956). The introduction of a satisfactory assay system for human prolactin (hPRL) led to the demonstration that chlorpromazine produces a rise in basal hPRL concentrations in normal subjects (Friesen, Guyda, Hwang, Tyson and Barbeau, 1972) and in patients receiving treatment with this drug (Apostolakis et al., 1972; Frantz et al., 1972). Most studies of the effects of phenothiazines in man have involved studies of oral administration of chlorpromazine and it was therefore decided to investigate the effects of fluphenazine decanoate (Modecate) a long acting depot phenothiazine preparation on basal hPRL concentrations and to follow the time course of changes in hPRL after a single injection. This background information was essential before designing investigations to study the effects of fluphenazine on hypothalamic/pituitary function.

METHODS

Patients

Basal hPRL concentrations were studied in 10 male chronic schizophrenics receiving long term treatment with fluphenazine. Ten male alcoholics were also studied before and after they received a single injection of fluphenazine (Modecate 12.5 mg). In addition, basal hPRL concentrations were studied in 17 healthy male control subjects. The time course of changes in hPRL concentrations was studied in 8 alcoholics (7 male, 1 female) before and after they received an injection of fluphenazine.

Procedure

All resting blood samples were taken from the various patients at 9-10 a.m. by venepuncture. The time course of changes in hPRL was determined by taking blood samples at approximately daily intervals before and for 10 days after the injection of fluphenazine. Significances of difference were examined using the students 't' test.

RESULTS

Chronic schizophrenics (Fig 8.I)

Basal hPRL concentrations were significantly increased ($P < 0.001$) in the group of schizophrenics receiving long term treatment. Prolactin levels were above the range of the controls (45-440 mu/l) in all the patients and three patients had levels greater than 1000 mu/l.

Alcoholics before and after treatment (Fig 8.I)

There was no significant difference between the basal hPRL concentrations of the controls and the alcoholics before treatment. The alcoholics showed a significant increase ($P < 0.02$) in hPRL levels after treatment and the levels were also significantly greater than the controls ($P < 0.002$). Nine of the ten patients had a rise in hPRL concentrations but there was considerable variation in the hPRL levels of individual patients.

Daily prolactin concentrations before and after treatment (Table 8.I)

Basal hPRL concentrations increased after the injection of fluphenazine in all the patients. Two patients showed

only minor changes, but the remaining six patients all showed a marked rise in hPRL concentrations. The peak concentrations occurred on the first day after the injection in one patient and on the 5th-6th day in the remaining seven patients.

FIG 8.I Prolactin concentrations (mu/l, mean ± S.E.M.) in 10 male schizophrenics receiving long term treatment with fluphenazine, in 10 male alcoholics before and after a single injection of fluphenazine (Modecate 12.5 mg) and the mean concentrations in 17 male control subjects.

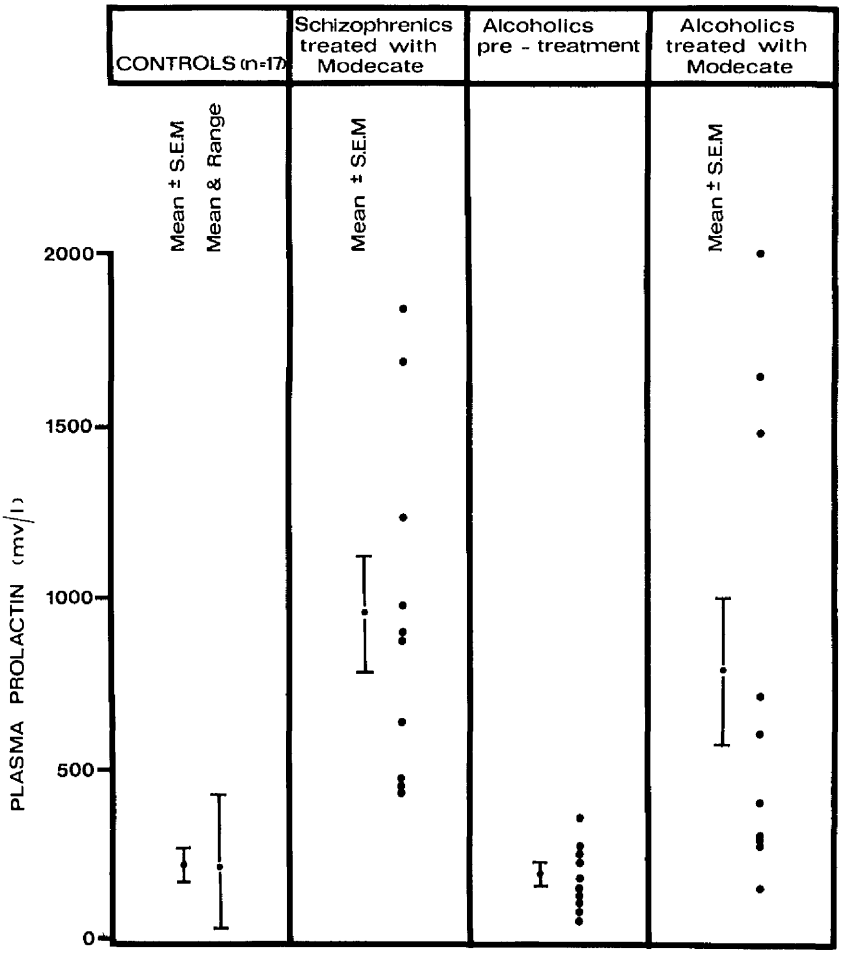

CONTROLS (n=17)
Schizophrenics treated with Modecate
Alcoholics pre - treatment
Alcoholics treated with Modecate
Mean ± S.E.M
Mean & Range
Mean ± S.E.M
Mean ± S.E.M
PLASMA PROLACTIN (mv/l)
2000
1500
1000
500
0

TABLE 8.1 Basal hPRL concentrations in 8 alcoholics before and after treatment with a single intramuscular injection of fluphenazine (Modecate 12.5 mg).

hPRL mu/1

Patients	Pre-treatment	Days after treatment									
	0	0	1	2	3	4	5	6	7	8	9	10
E.D.	320	280	370	320	400	400	480	360	400	320	-	440
H.McG	560	640	1160	920	840	1020	1300	700	700	740	740	680
T.A.	240	360	360	460	500	520	-	740	720	-	360	360
W.F.	260	280	640	560	440	380	440	-	520	560	350	300
A.McL	160	160	360	360	410	420	430	-	380	-	340	300
S.McG	440	500	1560	1400	1400	1240	1600	1500	1120	-	750	720
J.G.	280	320	640	640	620	720	740	-	580	-	420	420
A.C.	300	440	1500	1500	1960	2540	2760	-	1960	-	1660	1660

DISCUSSION

The results presented in this chapter demonstrate that hPRL concentrations are elevated in a proportion of patients receiving fluphenazine and are consistent with published findings for other phenothiazine derivatives (Apostolakis et al., 1972; Frantz et al., 1972; Beumont et al., 1974; Meltzer et al., 1976; Wiles et al., 1976). Release of hPRL from the pituitary is under inhibitory control from the hypothalamus and there is evidence that catecholamines inhibit hPRL release (Kamberi, 1973). Phenothiazines are known to block dopamine and noradrenaline receptors (Sourkes, 1975) and the ability of phenothiazines to promote elevation of hPRL concentrations may be due to blocking of the inhibitory effects of catecholamines on hPRL release.

In the present investigations not all the patients showed marked elevation of hPRL concentrations and those that did showed considerable variation after treatment with the same dose of fluphenazine. The magnitude of the rise in hPRL in response to equivalent oral doses of phenothiazines varies widely in schizophrenics (Meltzer et al., 1976) and the present results indicate that

there is also considerable variation in the hPRL response in non-psychotic patients. Serum chlorpromazine (CPZ) levels vary in patients receiving the same oral dose of CPZ and some of this variation is due to metabolism of CPZ by the intestinal wall during absorption (Curry, Marshall, Davis and Janovsky, 1970; Curry, 1971). However, this would not have been an important factor in the present studies as fluphenazine was administered by intramuscular injection. Prolactin levels are known to be related to CPZ levels (Kolakowska et al., 1975) and the variation in hPRL levels in patients reported in this chapter may be due to individual differences in metabolism of fluphenazine. A further possibility is that hypothalamic catecholaminergic systems have differing sensitivity to phenothiazines in different patients.

The neuroendocrine strategy of investigating pituitary hormone levels in relation to mental disease and phenothiazine drugs is complex but some indication of the involvement of dopamine metabolism is suggested. In particular measurement of hPRL levels may be useful in studying the extent of dopamine receptor blockade as this may be related to the development of symptoms of extrapyramidal syndrome as a side effect of phenothiazine

treatment. The assessment of the significance of daily hPRL concentrations is difficult in patients receiving daily oral doses of phenothiazines and the results presented in this chapter suggest that the prolactin response to an injection of fluphenazine may provide a useful method of measuring the response of individual patients to phenothiazines. The variation in hPRL concentration following the same dose of fluphenazine is of considerable interest and merits further investigation in relation to phenothiazine metabolism and the variation in response to other drugs and stimuli that elevate hPRL. The time course of changes in hPRL levels demonstrated that peak concentrations occurred 5-6 days after the injection in most patients and it was therefore decided to investigate the effect of fluphenazine on release of pituitary hormones by studying patients seven days after the injection of fluphenazine and the results of this study are reported in the following chapter.

SUMMARY

1. The effect of fluphenazine on basal hPRL concentrations was studied in 10 male schizophrenics receiving long term treatment, in 10 male alcoholics before and after treatment and in 8 alcoholics at daily intervals before and after treatment. The results were compared with basal hPRL concentrations in 17 healthy male controls. Investigations involved giving fluphenazine (Modecate 12.5 mg) by intramuscular injection and taking blood samples at appropriate intervals by venepuncture.

2. Basal hPRL concentrations were significantly increased in the schizophrenics. The alcoholics showed a significant rise in hPRL concentrations after the injection but there was considerable variation in hPRL levels of individual patients. Most patients showed marked elevation of daily hPRL levels with peak concentrations occurring 5-6 days after the injection.

3. The results demonstrate that fluphenazine elevates basal hPRL levels and the variation between levels of individual patients merits further investigation. Information on the changes in daily hPRL concentration after the injection was used to design further investigations

CHAPTER 9

EFFECT OF FLUPHENAZINE ON PITUITARY FUNCTION IN MAN

INTRODUCTION

Investigations in animals have demonstrated that phenothiazines alter hypothalamic endocrine functions (de Wied, 1967). In man phenothiazines are known to elevate hPRL concentrations (Beumont et al., 1974; Meltzer, 1976; Wiles et al., 1976 and previous chapter), but studies of hGH are less consistent and chlopromazine has been reported to depress both basal hGH concentrations and the response to hypoglycaemia (Sherman, Kim, Benjamin and Kolodny, 1971) while others have reported that this response is enhanced (Schimmelbusch, Muller and Scheps, 1971). It was decided to extend these studies and to investigate the effect of a long acting depot preparation of fluphenazine (Modecate) on hGH and hPRL responses to insulin hypoglycaemia.

METHODS

Subjects

Six male alcoholics mean age 44 years (range 33-53 years) who had given informed consent to the investigation were studied. All the patients admitted consuming relatively large amounts of alcohol on a regular basis and liver function tests on admission to hospital did not demonstrate any marked liver damage. The six alcoholics were selected from a larger group of nine patients on the basis of demonstrating an adequate hGH response to insulin hypoglycaemia when first investigated.

Procedure

The six patients were studied on two separate occasions. The first investigations were carried out 2-7 days after cessation of drinking. The patients then received a single intramuscular injection of fluphenazine (Modecate 12.5 mg) and were reinvestigated seven days later. In each investigation venous blood samples were withdrawn from a cannula in an antecubital vein before and at 10, 20, 25, 30, 35, 40, 45 and 60 minutes after the injection of soluble insulin (0.1 U/kg body weight) via the cannula.

Blood samples were analysed for glucose and plasma samples were analysed by radioimmunoassay technique for hGH and hPRL. Significances of difference were examined using the Mann-Whitney U-test for small samples (Mann & Whitney, 1947).

RESULTS

Blood glucose (Fig 9.I)

The rate of fall of blood glucose concentrations after the insulin injection was similar on both occasions before and after fluphenazine treatment. There was no significant difference in blood glucose concentrations at any time during the investigation before or after fluphenazine treatment. All the patients developed adequate hypoglycaemia (blood glucose < 2.2 mmol/l) for the hGH response.

Plasma hGH (Fig 9.I)

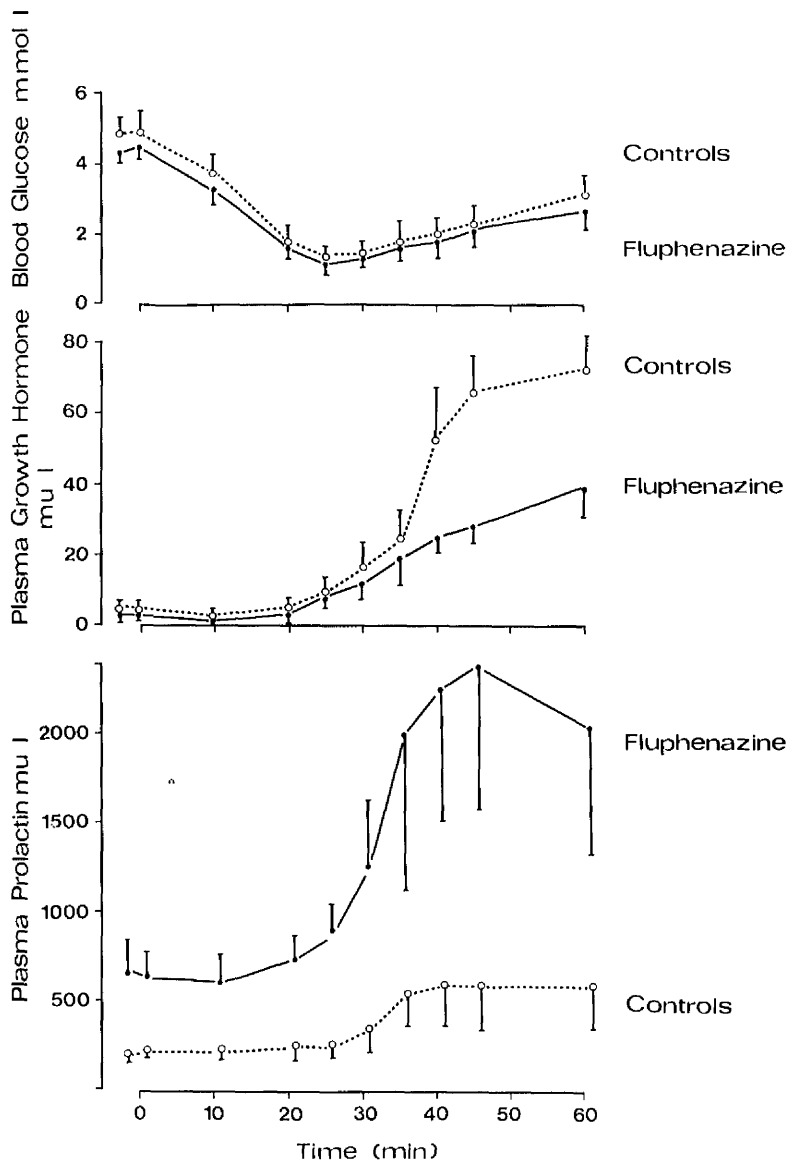
There was no significant difference between the basal hGH concentrations on the two occasions. Plasma hGH concentrations after fluphenazine treatment were significantly lower than the pre-treatment concentrations at 40, 45 and 60 min ($P < 0.05$, 0.01 and 0.02 respectively) after the insulin injection.

Plasma hPRL (Fig 9.I)

Basal hPRL concentrations were significantly increased ($P < 0.01$) after fluphenazine treatment. Insulin hypoglycaemia produced a small but consistent elevation in hPRL concentrations (peak response 567 mu/l). After treatment with fluphenazine a greater hPRL response occurred (peak response 2360 mu/l) and hPRL concentrations were significantly greater than pre-treatment concentrations at 10, 20, 25, 30, 35, 40, 45 and 60 minutes ($P < 0.01$, 0.01, 0.01, 0.03, 0.05, 0.03, 0.02 and 0.05

respectively). Although the hPRL responses were generally much greater after fluphenazine treatment, there was considerable variation between patients.

FIG 9.1 Blood glucose (mmol/l), plasma growth hormone (mu/l) and plasma prolactin (mu/l) in six patients during an insulin tolerance test (0.1 U/kg body weight) before and after treatment with fluphenazine (Modecate 12.5 mg).


Blood Glucose mmol l
6
4
2
0
Controls
Fluphenazine
Plasma Growth Hormone mu l
80
60
40
20
0
Controls
Fluphenazine
Plasma Prolactin mu l
2000
1500
1000
500
Fluphenazine
Controls
0
10
20
30
40
50
60
Time (min)

DISCUSSION

There is some disagreement about the effect of phenothiazines on hGH release in man. The effect of chlorpromazine on the hGH response to insulin hypoglycaemia has been studied by a number of investigators. Sherman et al., (1971) reported that this drug depressed both basal hGH concentrations and the response to hypoglycaemia, while Saldanha, Harvard, Bird and Gardner, (1972) found that the hGH response was lower but could not demonstrate a significant impairment. However, the observation of Schimmelbusch et al., (1971) that chlorpromazine treatment enhanced this response to hypoglycaemia in schizophrenics, is in marked contrast to these findings.

In the present investigation, the hGH response to insulin hypoglycaemia was significantly impaired in the patients after treatment with a single injection of fluphenazine (Modecate). This impaired hGH response is similar to that reported following oral administration of 200 mg of chlorpromazine for seven days (Sherman et al., 1971). Basal hPRL concentrations were found to be elevated after a single injection of fluphenazine (Modecate) and the results are consistent with the results presented in the previous chapter and with a number of reports on the effects of phenothiazines in man (Beumont et al., 1974; Meltzer, 1976; Wiles et al., 1976). Hypoglycaemia can produce a rise in hPRL concentrations but blood glucose concentrations lower than those reported in

this study are generally required for a consistent response (Noel et al., 1971). The patients before treatment showed a small rise in hPRL concentrations after the insulin injection but after treatment with fluphenazine a much greater, but variable, rise in hPRL concentrations occurred. The results suggest that a single injection of fluphenazine (Modecate 12.5 mg) has a marked effect on hypothalamic mechanisms controlling hGH and hPRL release.

Differences in hGH and hPRL responses might be produced by differences in the rate of fall of blood glucose concentrations, but there was no significant difference in blood glucose concentrations on the two occasions in our study. In addition, all the patients developed adequate hypoglycaemia (blood glucose $\leq$ 2.2 mmol/l) for the hGH response on both occasions. Phenothiazines are known to block dopamine and noradrenaline receptors (Anden, Carlsson and Haggendal, 1969; Sourkes, 1975) and dopaminergic and noradrenergic neurones are known to modulate hGH release (Martin, 1973). The impaired hGH response may be due to blockade by fluphenazine of hypothalamic dopamine or noradrenaline receptors mediating hGH release. Release of hPRL is under inhibitory control from the hypothalamus and there is evidence that dopamine and noradrenaline inhibit prolactin release (Zacur, Foster and Tyson, 1976). The marked rise in basal hPRL concentrations after fluphenazine

treatment and the increased response to hypoglycaemia suggests that hPRL inhibitory control mechanisms have been altered by this drug.

The results of other studies reported in this thesis (chapters 5 and 6) demonstrate that there is an impaired hGH response to insulin induced hypoglycaemia in some alcoholics a few days after cessation of drinking. In the present investigation the six alcoholics studied all shared a normal hGH response to insulin hypoglycaemia when investigated for the first time, and it is therefore unlikely that the present results are due to impaired hypothalamic/pituitary function resulting from chronic alcohol abuse. The results presented in this chapter demonstrate that a single intramuscular injection of fluphenazine has a marked effect on hGH and hPRL release mechanisms. Further investigation will be required to determine if this is an important effect during long term treatment and to assess the extent to which the release of other pituitary hormones is impaired.

SUMMARY

1. The growth hormone (hGH) and prolactin (hPRL) response to insulin induced hypglycaemia was studied in six alcoholics on two occasions before and after treatment with a single intramuscular injection of fluphenazine (Modecate). On both occasions blood samples were taken at intervals before and after the injection of soluble insulin (0.1U/kg body weight). The patients were investigated on the first occasion, 2-7 days after cessation of drinking and they all demonstrated an adequate hGH response. They then received an injection of fluphenazine (Modecate 12.5 mg) and were reinvestigated one week later.

2. The hGH response to hypoglycaemia was significantly impaired after treatment with fluphenazine. Basal hPRL concentrations were significantly increased and increased concentrations of hPRL in response to hypoglycaemia occurred after treatment.

3. The results presented in this chapter demonstrate that a single injection of fluphenazine (Modecate 12.5 mg) has a marked effect on hypothalamic/pituitary mechanisms controlling hGH and hPRL release.

CHAPTER 10

GROWTH HORMONE AND PROLACTIN RELEASE IN RESPONSE TO INTRAVENOUS ADMINISTRATION OF CLONIDINE

INTRODUCTION

It is well established that release of pituitary hormones is under the control of the hypothalamus. Catecholaminergic neurones have been demonstrated in the basal hypothalamus and most of these neurones appear to be dopaminergic (Jonson, Fuxe and Hokfelt, 1972). Evidence that catecholamines can modulate release of hGH and hPRL includes the observation that L-dopa, the precursor of dopamine and noradrenaline, produces elevation of hGH and suppresses hPRL in man (Boyd et al., 1970; Eddy, Jones, Chakmakjian and Silverthorne, 1971). It has been proposed that L-dopa increases both dopamine and noradrenaline levels in the brain and these results are consistent with a regulatory role for either or both of these monamines (Eddy et al., 1971). There is considerable evidence for the involvement of dopamine in the regulation of hGH and hPRL release and some of this evidence has already been discussed (see chapter 7). However, the role of noradrenaline in the regulation of release of these hormones remains uncertain.

The drug clonidine selectively stimulates central noradrenaline receptors without affecting dopamine or serotonin receptors (Anden, Corrodi, Fuxe, Hokfelt, Rydin and Svensson, 1970). It was therefore decided to investigate the effect of intravenous administration of clonidine on hGH and hPRL release and theresults are presented in this chapter.

METHOD

Subjects

Six normal healthy male subjects aged 23-35 years (mean 31 years) were studied. The investigations were carried out in the morning after an overnight fast.

Procedure

Blood samples were collected before and at 10, 20, 30, 40, 50, 60, 90, and 120 minutes after an injection of 0.1 mg clonidine HCl (Catapress, Boehringer Ingelheim Ltd) in 20 ml of saline over 10 minutes.

Blood samples were analysed for glucose and plasma samples for insulin, hGH, hPRL and FFA. Significance of difference was examined using the Mann-Whitney non-parametric U-test for small samples (Mann and Whitney, 1947).

RESULTS

Blood glucose (Fig 10.I)

Injection of clonidine was associated with a significant increase ($P < 0.05$) in blood glucose concentrations at 10 and 20 minutes. Thereafter, blood glucose concentrations returned to basal pre-injection levels.

Plasma insulin (Fig 10.I)

Plasma insulin concentrations showed a progressive fall throughout the investigation after the injection and were significantly lower than basal concentrations at 90 and 120 minutes ($P < 0.05$ and $P < 0.02$ respectively).

Plasma hGH (Fig 10.II)

Growth hormone concentrations increased after the injection of clonidine and reached peak concentrations at 40 minutes. The concentrations of hGH were significantly greater ($P < 0.002$) than basal concentrations at 20, 30, 40, 50 and 60 minutes.

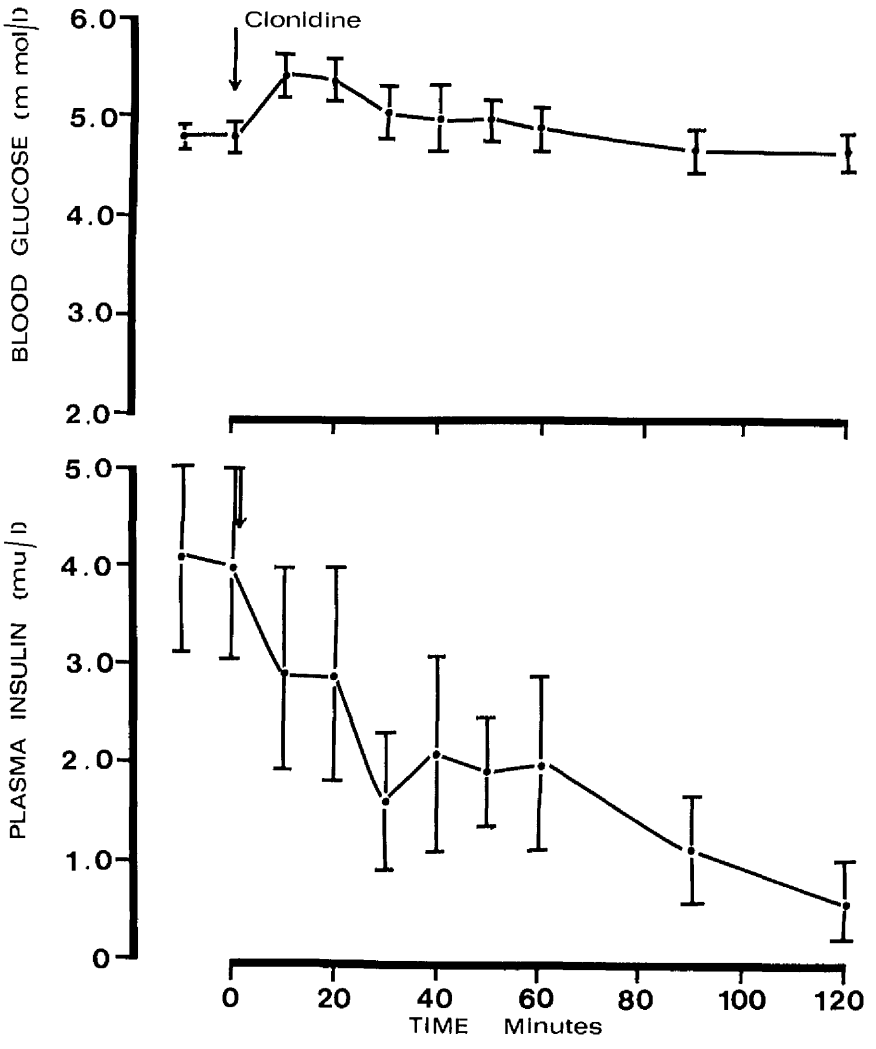
Plasma hPRL (Fig 10.II)

Concentrations of hPRL decreased after the injection of clonidine and were significantly lower ($P < 0.05$) than basal concentrations at 50, 60 90 and 120 minutes.

Plasma free fatty acids

There was no significant change in FFA concentrations after the injection of clonidine.

FIG. 10.I Blood glucose (mmol/l, mean $\pm$ SEM) and plasma insulin concentrations (mu/l, mean $\pm$ SEM) in six normal healthy male subjects before and after intravenous administration of clonidine (0.1 mg).


Clonidine
BLOOD GLUCOSE (m mol/l)
6.0
5.0
4.0
3.0
2.0
PLASMA INSULIN (mu/l)
5.0
4.0
3.0
2.0
1.0
0
0
20
40
60
80
100
120
TIME Minutes

DISCUSSION

The results presented in this chapter demonstrate that clonidine stimulates hGH release and this observation is consistent with the results of other investigators (Lal et al., 1975) and with the evidence for adrenergic regulation of hGH release (Blackard and Heidingsfelder, 1968). In the present investigation clonidine suppressed hPRL concentrations and this is not in agreement with the report of Lal and co-workers (1975) but is consistent with the suppression of prolactin levels reported in rats following an injection of noradrenaline into the third ventricle of the brain (Kamberi, 1973).

Growth hormone release is known to follow a fall in FFA concentrations (Hartog et al., 1967) or a fall in blood glucose (Hunter et al., 1965a). However, there was no indication of a fall in either FFA or blood glucose concentrations in the present study. Blood glucose concentrations increased after clonidine administration in the present investigation and it is possible that this may have led to some antagonism of the hGH response as hyperglycaemia is known to antagonise hGH release (Mims, Scott, Modebe and Bethune, 1973; Ettigi, Lal, Martin and Friesen, 1975). Furthermore, there is evidence that adrenergic stimulation can inhibit insulin release. This evidence

includes the observations that adrenaline infusion causes a fall in insulin levels and that infusion of adrenaline and the α-adrenergic blocking agent, phentolamine, produces a rise in insulin levels, (Robertson and Porte, 1973). The present observation that clonidine, a drug known to have α-adrenergic stimulating properties, produced significant lowering of insulin levels is further evidence for an α-adrenergic inhibitory influence on insulin release. The increase in blood glucose after clonidine may be due to α-adrenergic stimulation of glycogenolysis or gluconeogenesis (Day, 1975), although decreased insulin release may also have been an important factor.

Maj, Baran, Grabonska and Snowinska (1973) have proposed that clonidine, in addition to stimulating noradrenergic receptors, may also inhibit serotonin release. However, the observation that 5-HTP, the precursor of serotonin, elevates hGH (chapter 11) does not support this suggestion. The possibility that the suppression of hPRL by clonidine does involve such a mechanism requires further investigation.

The results reported in this chapter suggest that hypothalamic noradrenergic receptors regulate hGH and hPRL release. Therefore, because of the evidence for the involvement of noradrenaline the results of studies involving non-specific drugs or precursors, e.g. phenothiazines and L-dopa, cannot

be interpreted as reflecting an action of dopamine alone. The present results demonstrate a significant and reproducible action of clonidine on hGH and hPRL release and it was therefore possible to use a similar investigation to study noradrenergic regulation of neuroendocrine responses in patients.

SUMMARY

1. Growth hormone and hPRL concentrations were investigated in six normal healthy male subjects before and after the injection of clonidine (0.1 mg).

2. Clonidine produced significant elevation of blood glucose shortly after the injection and produced significant suppression of insulin concentrations. These observations are consistent with the known α-adrenergic stimulating action of clonidine.

3. Growth hormone concentrations were significantly increased and hPRL concentrations significantly reduced after the injection of clonidine. The results suggest that hypothalamic noradrenergic receptors regulate hGH and hPRL release and clonidine may therefore be a useful drug in the investigation of noradrenergic regulation of neuroendocrine responses.

CHAPTER 11

THE EFFECT OF 5-HYDROXYTRYPTOPHAN ON GROWTH HORMONE AND PROLACTIN RELEASE

INTRODUCTION

The mammalian hypothalamus contains noradrenergic, dopaminergic and serotonergic neurones (Fuxe and Hokfelt, 1969). A number of studies have demonstrated that dopamine and noradrenaline releasing neurones play an important role in regulating hGH and hPRL release (Blackard and Heidingsfelder, 1968; Boyd et al., 1970; Lal et al., 1975; and chapters 7 and 10), but the role of serontonergic neurones is uncertain. The precursor of serotonin, 5-hydroxytryptophan (5-HTP) has been shown to cross the blood brain barrier and to be converted into serotonin in animals (Corrodi, Fuxe and Hokfelt, 1967). Oral administration of 5-HTP in man produces an elevation of hGH and hPRL concentrations (Imura et al., 1973). It was decided to investigate the effect of an intravenous dose of 5-HTP on hGH and hPRL concentrations and thereby determine if a suitable investigation could be designed to study serotonergic regulation of neuroendocrine responses in patients.

METHODS

Subjects

Five normal healthy subjects (4 males, 1 female) aged 28-40 years (mean 34 years) were studied. The subjects were investigated in the morning after an overnight fast and remained at rest throughout the investigation.

RESULTS

Plasma hGH (Fig 11.I)

Growth hormone concentrations increased after the infusion of 5-HTP. The peak concentration occurred 40 minutes after the infusion and concentrations were significantly greater than basal pre-infusion levels at 20, 30, 40, 50 and 60 minutes ($P < 0.05$, 0.05, 0.005, 0.005 and 0.05 respectively).

Plasma hPRL (Fig 11.I)

Prolactin concentrations increased after 5-HTP infusion reaching a peak level at 40 minutes. Concentration of hPRL were significantly greater than basal pre-infusion levels at 20, 30, 40 and 50 minutes ($P < 0.05$, 0.02, 0.005 and $P < 0.05$ respectively).

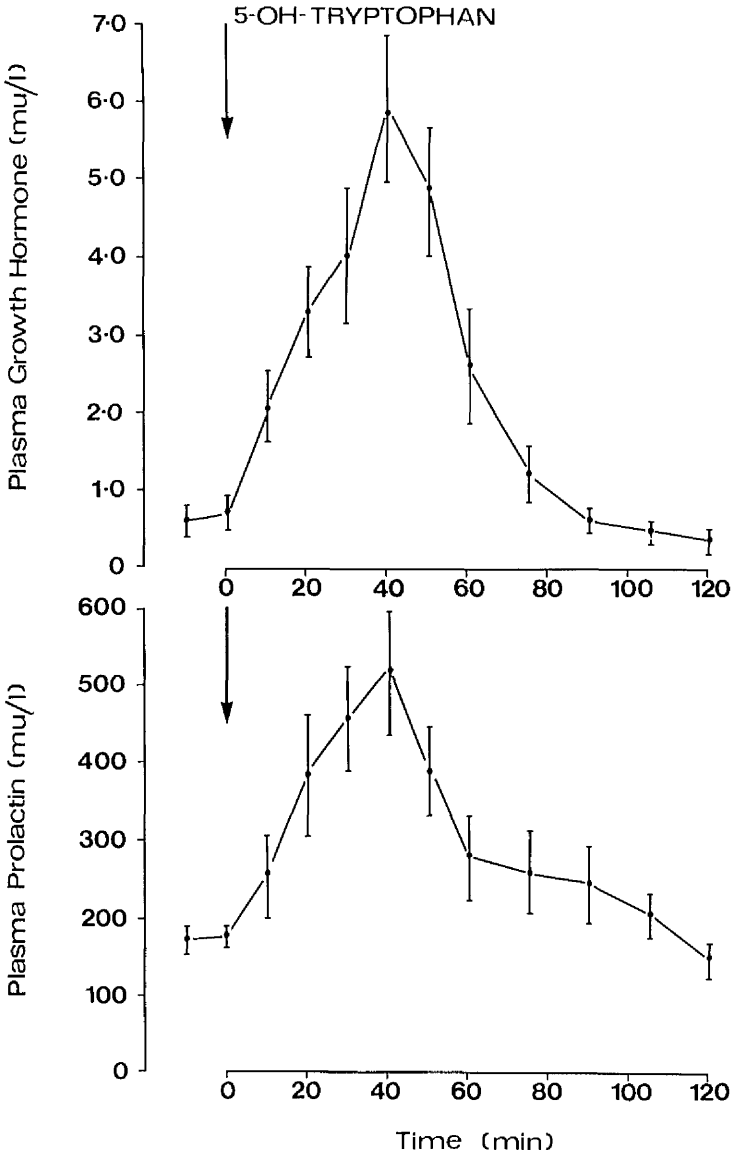
Blood glucose

There was no significant change in blood glucose concentrations after the infusion of 5-HTP.

Plasma FFA

Plasma FFA concentrations did not show any significant change after the infusion of 5-HTP.

FIG. 11.I Plasma growth hormone and prolactin concentrations (mu/l, mean $\pm$ SEM) in five normal healthy subjects before and after intravenous infusion of 5-hydroxy-L-tryptophan (20 mg).


5-OH-TRYPTOPHAN
Plasma Growth Hormone (mu/l)
7·0
6·0
5·0
4·0
3·0
2·0
1·0
0
0
20
40
60
80
100
120
Plasma Prolactin (mu/l)
600
500
400
300
200
100
0
0
20
40
60
80
100
120
Time (min)

DISCUSSION

The results described in the present chapter demonstrate that intravenous infusion of 5-HTP (20 mg) produced a significant rise in hGH and hPRL concentrations. The response consisted of a well defined peak forty minutes after the infusion. Studies of oral administration of 5-HTP (150-200 mg) also demonstrate elevation of hGH and hPRL but the response is less well defined although the overall maximum increase associated with the larger dose of 5-HTP was greater, (Imura et al., 1973; Kato et al., 1973).

The mechanism by which 5-HTP elevates hGH and hPRL is not known. The absence of any side effects or symptoms of stress in the subjects in the present investigation suggests that a non-specific stress effect is not involved. Growth hormone release is known to follow a fall in FFA concentrations (Hartog et al., 1967) and hGH and hPRL release to follow a fall in blood glucose (Hunter et al., 1965a; Noel et al., 1971) but there was no significant change in either FFA or blood glucose after the infusion in the present investigation. Elevation of brain serotonin levels has been associated with 5-HTP administration in animals (Corrodi et al., 1967) and L-tryptophan, the precursor of 5-HTP, also elevates brain serotonin levels in animals (Ferustrom and Wurtman, 1971) and increases hPRL levels in man (Macindoe and Turkington, 1973). Further evidence that the response to 5-HTP is mediated by serotonin comes from the observation that the serotonin receptor blocker cyproheptadine

inhibits the hGH and hPRL response to 5-HTP (Nakai, Imura, Sakurai, Kurahachi and Yoshimi, 1973; Kato et al., 1973). An alternative explanation to direct involvement of serotonin might involve an action of serotonin on catecholamine regulation of hGH and hPRL release and there is evidence that serotonin can displace dopamine from dopaminergic neurones (Fuxe, Butcher and Engel, 1971). However, although such a mechanism could produce an elevation of hGH dopamine is reported to inhibit hPRL release (Frohman and Stachura, 1975) whereas in the present investigation 5-HTP elevated hPRL.

The results presented in this chapter confirm the findings of other research workers (Imura et al., 1973) and suggest that serotonin may be involved in the regulation of hGH and hPRL release. However, further investigations are required to determine if there is direct involvement of serotonergic neurones or whether the effect of serotonin involves alteration of catecholamine levels. The infusion of 5-HTP in the present study produced well defined changes in hGH and hPRL concentrations and intravenous adminstration of 5-HTP may provide a useful method of investigating serotonergic regulation of pituitary hormone release.

SUMMARY

1. Growth hormone and hPRL concentrations were measured in five normal healthy subjects before and after the infusion of 5-hydroxy-l-tryptophan (5-HTP, 20 mg).

2. The intravenous administration of 5-HTP was associated with significant elevation of both hGH and hPRL concentrations. Changes in hGH and hPRL concentrations consisted of well defined responses with peak concentrations occurring 40 minutes after intravenous administration of 5-HTP. There was no significant change in either blood glucose or FFA concentrations after 5-HTP administration.

3. The results support the suggestion that serotonin is involved in the regulation of both hGH and hPRL release.

CHAPTER 12

THE EFFECT OF SOMATOSTATIN ON METABOLIC AND HORMONAL CHANGES DURING AND AFTER EXERCISE

INTRODUCTION

Somatostatin is a cyclic tetradecapeptide originally isolated from ovine hypothalamus and postulated to be a hypothalamic growth hormone release inhibiting hormone (Brazeau et al., 1973). Growth hormone responses to exercise (Hansen et al., 1973), arginine infusion and L-dopa administration (Siler et al., 1973) are inhibited by somatostatin. In addition to its effects on hGH release, somatostatin has been reported to inhibit release of insulin (Chideckel et al., 1973), glucagon (Gerich et al., 1974) and prolactin (Yen et al., 1974).

It has been suggested that hGH may play an important role in facilitating mobilisation of depot fat during exercise (Hunter et al., 1965b; Greenwood and Landon, 1966). The present investigations were carried out to examine the effect of somatostatin during exercise in normal subjects. Suppression of hGH and other hormones allows their effects on mobilisation and utilisation of fatty acids and other metabolic fuels during exercise to be studied.

METHODS

Subjects

Six healthy male subjects mean age 29 years (range 23-35 years) were investigated on two occasions after an overnight fast.

Procedure

The exercise capacity of each subject was assessed several days before the first investigation by means of an increasing work test (Spiro, Juniper, Bowman and Edwards, 1974). The subjects exercised on a bicycle -ergometer at a workload of 200 kpm for 2 minutes and the workload was increased by 200 kpm every 2 minutes until they were unable to continue.

The six subjects were subsequently investigated on two occasions during and after exercise at 70% of maximal exercise capacity for 30 minutes. On the first occasion each subject received an injection of somatostatin (200 μg) immediately before and an infusion of somatostatin (300 μg in 30 ml saline at 1 ml/min) from five minutes after the start of exercise until five minutes after the completion of exercise. A control exercise test was performed by all the subjects one week later when no somatostatin was received.

Venous blood samples were taken from a cannula in an antecubital vein before exercise, at five minute intervals during exercise and at 5, 15, 30, 45, 60 and 90 minutes after exercise. Blood samples were analysed for lactate, pyruvate, glycerol, acetoacetate, 3-hydroxybutyrate and glucose. Plasma samples were analysed for FFA, insulin, glucagon, hGH and hPRL. The significance of differences was examined using the Mann-Whitney non-parametric U-test for small samples (Mann and Whitney, 1947). Synthetic cyclic somatostatin was supplied by Ayerst Laboratories Limited.

RESULTS

Blood lactate and pyruvate

Basal blood lactate and pyruvate were similar on both occasions. Blood lactate concentrations increased during exercise and returned towards resting concentrations after exercise on both occasions (Fig. 12.I). The changes in pyruvate concentrations were similar. There was no significant difference between concentrations of either lactate or pyruvate at any time during the investigation on both occasions.

Plasma FFA (Fig. 12.I)

No significant difference was observed in resting FFA concentrations on the two occasions. There was a rise in FFA concentrations towards the end of exercise on both occasions. Concentrations of FFA tended to be lower during exercise with somatostatin than in the control investigation but the difference was not significant.

Blood glycerol (Fig 12.I)

Resting concentrations of blood glycerol were not significantly different on the two occasions. Exercise was associated with an increase in blood glycerol concentrations on both occasions. Glycerol concentrations during exercise with somatostatin tended to be greater

than the corresponding control values but the difference was not significant.

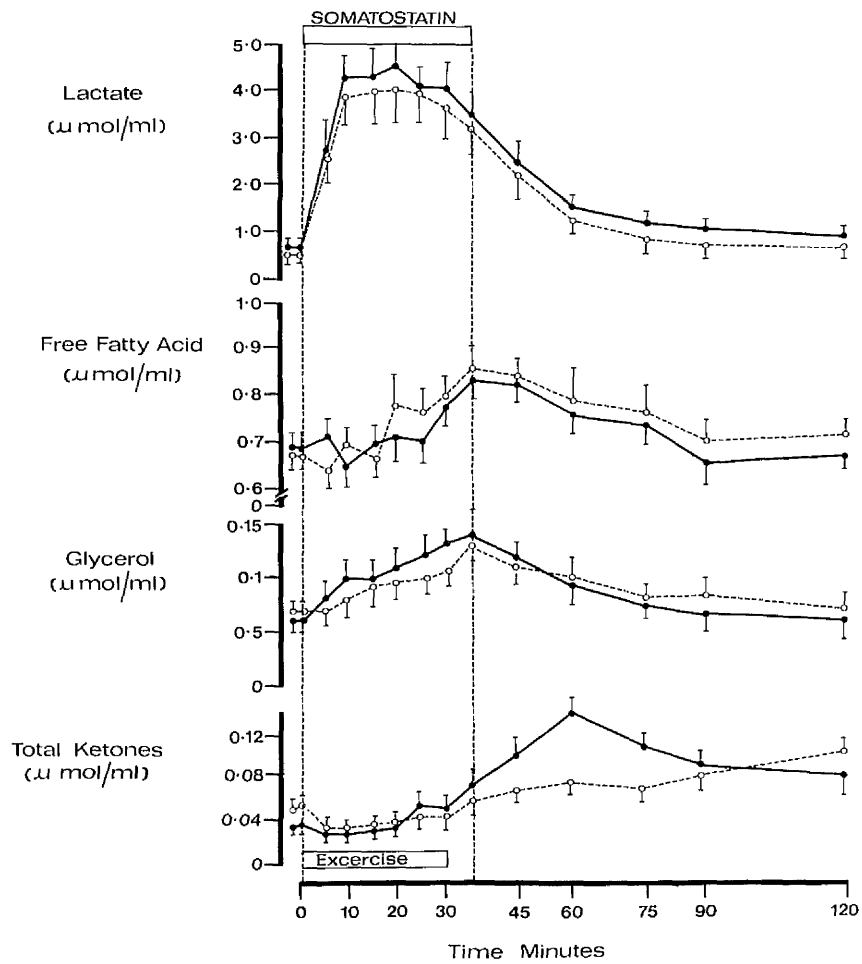
Total blood ketones (Fig 12.I)

The concentrations of total blood ketones (acetoacetate + 3-hydroxybutyrate) were similar at rest on both occasions. There was no significant change in ketone concentrations during exercise on both occasions. Ketone concentrations increased after exercise on both occasions but in the investigation with somatostatin the rise was greater after exercise and concentrations at 45, 60 and 75 minutes were significantly greater than the corresponding control concentrations ($P<0.05$, 0.01 and 0.05 respectively).

Blood glucose (Fig. 12.II)

There was no significant difference in basal blood glucose concentrations. There was a fall in blood glucose concentrations during exercise with somatostatin and concentrations at 15 minutes were significantly lower than in the control investigation ($P<0.05$). After exercise with somatostatin blood glucose concentrations increased and concentrations at 75 minutes were significantly greater than control values ($P<0.05$).

FIG. 12.I Blood lactate, plasma free fatty acids, blood glycerol and total ketone (acetoacetate + 3-hydroxybutyrate) concentrations (umol/ml, mean ± SEM) in six normal subjects during and after exercise with the infusion of somatostatin (O———O) and in a control investigation (O------O).


SOMATOSTATIN
Lactate
(μ mol/ml)
5·0
4·0
3·0
2·0
1·0
0
Free Fatty Acid
(μ mol/ml)
1·0
0·9
0·8
0·7
0·6
0
Glycerol
(μ mol/ml)
0·15
0·1
0·5
0
Total Ketones
(μ mol/ml)
0·12
0·08
0·04
0
Excercise
0
10
20
30
45
60
75
90
120
Time Minutes

Plasma insulin (Fig. 12.II)

Plasma insulin concentrations were similar at rest and decreased during exercise in both investigations. Concentrations of insulin during exercise with somatostatin were lower than control values but the differences were not significant. After exercise plasma insulin levels increased on both occasions but in the investigation with somatostatin the increase in insulin concentrations did not occur until after completion of the somatostatin infusion. The insulin values at 35 minutes with somatostatin were significantly lower than the corresponding control concentrations ($P<0.05$).

Plasma glucagon (Fig. 12.II)

There was no significant difference in basal glucagon concentrations. Glucagon concentrations increased towards the end of exercise in the control investigation whereas somatostatin infusion was associated with significantly lower glucagon concentrations at 5, 10, 15, 20, 25 and 30 minutes during exercise ($P<0.01$, 0.005, 0.005, 0.01, 0.02 and 0.02 respectively). In the control investigation glucagon concentrations continued to rise initially after exercise but this rise did not occur until after completion of the somatostatin infusion in the other

investigation and the glucagon concentration at 35 minutes was significantly lower ($P<0.05$) than the corresponding control values.

Plasma growth hormone (Fig. 12.III)

Basal hGH concentrations were similar on the two occasions. Growth hormone concentrations increased during exercise in the control investigation but the administration of somatostatin produced complete suppression of the hGH response and concentrations at 15, 20, 25, 30 and 35 minutes were significantly lower than corresponding control concentrations ($P<0.05$, 0.005, 0.001, 0.001 and 0.001 respectively). Growth hormone concentrations increased after completion of the somatostatin infusion whereas they decreased in the control investigations. Concentrations at 75 and 90 minutes in the investigation with exercise and somatostatin were significantly greater than the corresponding control concentrations ($P<0.05$).

Plasma prolactin (Fig. 12. III)

There was no significant difference in basal hPRL concentrations. Exercise produced a small and rather variable rise in hPRL concentrations on both occasions and hPRL concentrations during and after exercise were not significantly different on the two occasions.

FIG. 12.II Blood glucose (mmol/l, mean ± S.E.M.) plasma insulin (mu/l) and plasma glucagon (Pmol/l) in six normal subjects during and after exercise with the infusion of somatostatin (●——●) and in a control investigation (○-----○).

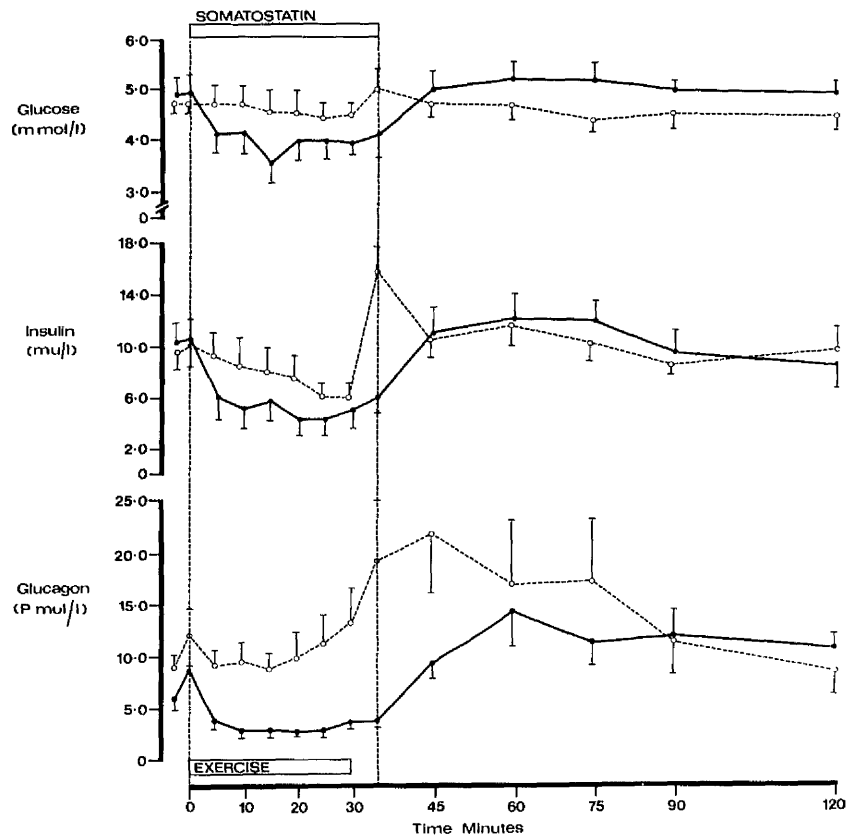
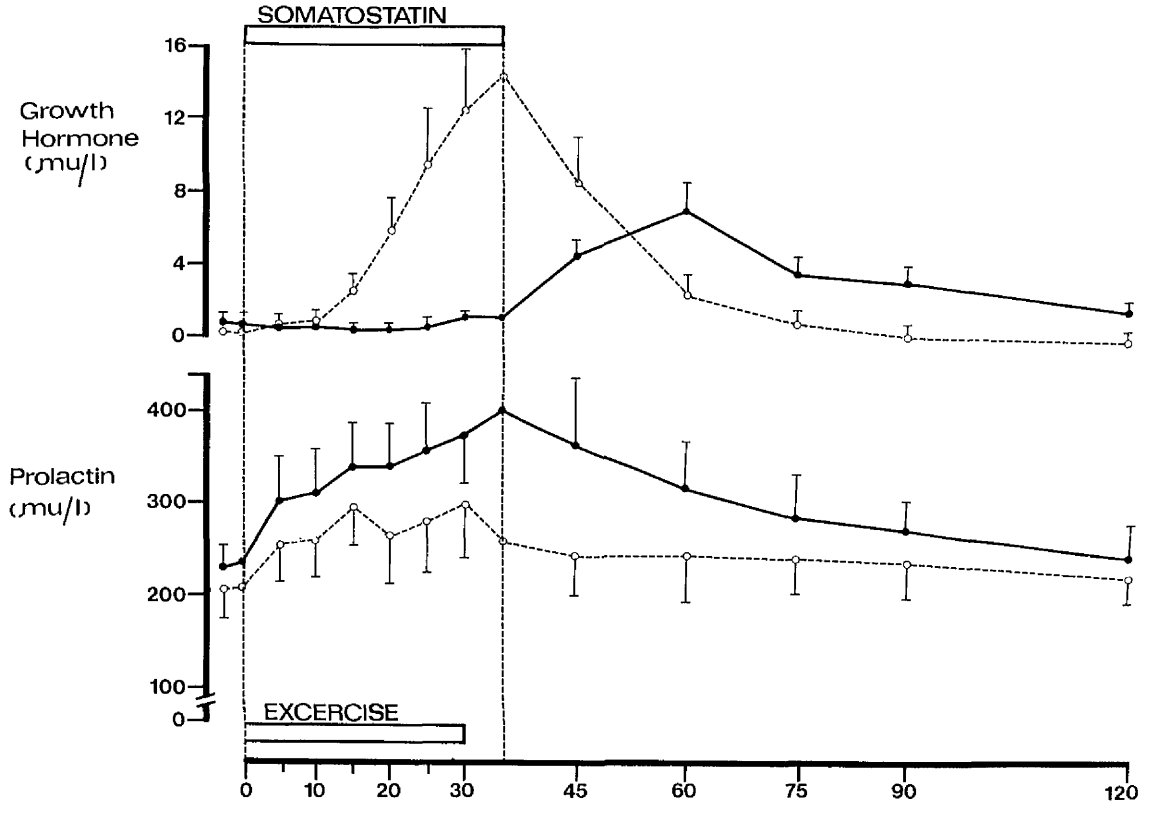

SOMATOSTATIN
Glucose (m mol/l)
6·0
5·0
4·0
3·0
0
Insulin (mu/l)
18·0
14·0
10·0
6·0
2·0
0
Glucagon (P mol/l)
25·0
20·0
15·0
10·0
5·0
0
EXERCISE
0
10
20
30
45
60
75
90
120
Time Minutes

FIG. 12.III Plasma growth hormone and prolactin concentrations (mu/l, mean ± SEM) in six normal subjects during and after exercise with the infusion of somatostatin (●———●) and in a control investigation (O------O).


SOMATOSTATIN
Growth Hormone (mu/l)
16
12
8
4
0
Prolactin (mu/l)
400
300
200
100
0
EXCERCISE
0
10
20
30
45
60
75
90
120

DISCUSSION

Metabolic and hormonal responses to exercise are reported to vary with work levels and fitness (Bloom et al., 1976). In the present study the same subjects performed an identical exercise test at 70% of maximal exercise capacity on the two occasions and it is unlikely that differences in metabolic or hormonal responses were related to differences in fitness.

Blood lactate concentrations increase during exercise because of anaerobic metabolism within muscle cells: cytoplasmic glycolysis exceeding mitochondrial oxidative capacity, (Keul et al., 1967). The present observation that blood lactate and pyruvate concentrations during exercise were not altered by somatostatin infusion suggests that somatostatin does not alter glycolytic activity in working muscle.

Free fatty acids and ketones, produced by oxidation of FFA in the liver, are utilised as a fuel by working muscle. In the present studies exercise produced similar elevations of both FFA and glycerol concentrations on both occasions and this suggests that somatostatin did not alter fat mobilisation or utilisation during exercise. The greater rise in ketone concentrations immediately after exercise with somatostatin suggests that there was greater hepatic

oxidation of FFA. However, there was little evidence of increased lipolysis or delayed re-esterification of FFA after exercise with somatostatin in these investigations.

In the control investigation blood glucose concentrations showed little change during exercise and this is consistent with the observation that glucose is not a major fuel of working muscle until muscle glycogen concentrations fall (Hermansen, Pruett, Osnes and Giere, 1970). However, infusion of somatostatin during exercise produced a marked fall in blood glucose concentrations in the present investigation. This observation is consistent with a number of reports that somatostatin induces hypoglycaemia (Koerker et al., 1974; Alford et al., 1974; Christensen et al., 1974; DeVane et al., 1974; Gerich et al., 1974). Somatostatin infusion in the present investigation showed greater suppression of insulin during exercise and inhibition of the glucagon response to exercise (Bottger, Schlein, Faloona, Knochel and Unger, 1972) and these observations are in agreement with the reported inhibitory action of somatostatin on insulin (Chideckel et al., 1973) and glucagon release (Gerich et al., 1974). The hypoglycaemic effect of somatostatin has been reported to be associated with inhibition of hepatic glucose production (Blauth, Sonksen, Tompkins and

Bloom, 1977). The decreased blood glucose concentrations associated with somatostatin infusion in the present study may therefore be due to decreased hepatic glucose production and this may be related to inhibition of glucagon release as both glucagon and glucose concentrations increased on cessation of the infusion of somatostatin.

In the present investigation the hGH response to exercise was completely suppressed by administration of somatostatin. It has been suggested that hGH may play an important role in the mobilisation of fat during exercise (Hunter et al., 1965b; Greenwood and Landon, 1966). This suggestion is weakened by results of studies in patients with hypopituitarism who fail to show an hGH response but have increased FFA and glycerol levels during exercise (Johnson, Rennie, Walton and Webster, 1971) and by the present observation that suppression of the hGH response was not associated with an alteration of FFA or glycerol levels. Growth hormone concentrations increased on completion of the somatostatin infusion in the present investigation. This rebound in hGH after somatostatin has been observed by other investigators (Blauth et al., 1977) and it is likely that this may be related to the fall in blood glucose concentrations during the somatostatin infusion as hypoglycaemia is a potent

stimulus for hGH release (Roth et al., 1963). A small but significant fall in hPRL concentrations has been reported to follow somatostatin infusion (Yen et al., 1974) but there was no evidence for suppression of hPRL release in the present investigation.

Somatostatin infusion suppressed the hGH and glucagon responses to exercise and produced greater suppression of insulin during exercise in the present study. Reduced glucose concentrations during exercise were probably related to inhibition of glucagon release by somatostatin but there was no evidence for any alteration of glycolysis or fat mobilisation or utilisation during exercise with somatostatin.

SUMMARY

1. The effect of intravenous somatostatin on blood levels of metabolites and hormones has been examined in normal subjects who performed a 30 minute period of bicycle exercise at 70% maximal exercise capacity. The results have been compared with control studies in the same subjects.

2. Measurements were made of blood levels of lactate, pyruvate, glucose, free fatty acids, glycerol, aceto-acetate, 3-hydroxybutyrate, insulin, glucagon, growth hormone (hGH) and prolactin.

3. Growth hormone and glucagon release was suppressed during exercise with somatostatin and there was a subsequent elevation during recovery. There was slight post exercise depression of insulin, but no alteration of plasma prolactin secretion.

4. Blood glucose was reduced during exercise with somatostatin and increased during recovery. The elevation of ketone bodies after exercise was greater in the investigation with somatostatin but there was no significant changes in other metabolites.

5. Somatostatin although causing inhibition of hGH release appears to have no significant effect upon fatty acid mobilisation during exercise and the results suggest that the hypoglycaemic action of somatostatin is related to glucagon suppression.

CHAPTER 13

GROWTH HORMONE RESPONSE TO INSULIN INDUCED HYPOGLYCAEMIA IN HUNTINGTON'S CHOREA

INTRODUCTION

Huntington's chorea is an inherited disorder associated with neuronal degeneration of the basal ganglia and cerebral cortex. The symptoms include dementia and involuntary movements or chorea which usually appear in middle life. Neuropathological evidence of hypothalamic involvement has been demonstrated (Bruyn 1968, 1973; Klintworth, 1973). Many patients with Huntington's chorea have weight loss and excessive sweating and this has been attributed to altered hypothalamic function (Bruyn, 1973). Abnormal hGH secretion has been reported in patients with the disorder in response to glucose loading (Podolsky, Leopold and Sax, 1972) and earlier hGH release has been demonstrated to follow insulin induced hypoglycaemia (Keogh et al., 1976; Phillipson and Bird, 1976). Keogh and co-workers (1976) suggested that studies of hGH release may be of value in the diagnosis of the disorder and an opportunity arose to study the response in four patients who were suspected of having Huntington's chorea. Evidence of an earlier release of hGH in these patients would help to confirm the diagnosis.

METHODS

Patients

Four patients with chorea were investigated. Two of the patients had a positive family history of Huntington's

chorea but at the time of investigation this was not known for the other two patients. The patients, (2 male, 2 female) mean age 56 years (range 47-68), were investigated after an overnight fast. None of the patients were receiving any medication at the time of the investigation. In addition 10 healthy controls (5 males, 5 females) mean age 42 years (range 32-57) were also studied.

Procedure

Blood samples were taken from a cannula in an antecubital vein before and at 10, 20, 25, 30, 35, 40, 45 and 60 minutes after the injection of insulin (0.1 U/kg body weight) via the cannula. Blood samples were analysed for glucose and plasma samples for hGH. Significance of differences were examined using the Mann-Whitney non-parametric U-test for small samples (Mann and Whitney, 1947).

RESULTS

Blood glucose (Table 13.I)

There was no difference in basal blood glucose concentrations between patients and controls. All the patients developed adequate hypoglycaemia for the hGH response (blood glucose 2.2 mmol/l) and there was no significant difference in blood glucose concentrations between patients and controls at any time after the injection of insulin.

Plasma growth hormone (Table 13.II)

Basal hGH concentrations of patients were not significantly different from controls. Peak hGH concentrations were similar in both groups but the rise in hGH concentrations in the patients was earlier than in the controls and hGH concentrations of the patients were significantly greater than the controls at 30 and 35 minutes.

TABLE 13.I Blood glucose concentrations (mmol/l) before and after the injection of insulin (0.1 U/kg body weight) in 4 patients with Huntington's chorea and the mean concentrations in 10 control subjects.

	blood glucose mmol/l									
	Time (min)									
Patients	0	0	10	20	25	30	35	40	45	60
1	4.4	4.3	3.3	1.7	1.3	1.1	1.4	1.6	1.5	1.5
2	4.6	4.4	3.8	1.8	0.9	1.2	2.0	2.2	2.3	2.0
3	5.3	5.6	5.0	2.8	1.9	1.8	1.7	2.0	1.9	2.9
4	4.6	4.4	3.8	1.8	0.9	1.2	2.0	2.2	2.3	2.2
MEAN	4.7	4.7	4.0	2.0	1.3	1.3	1.8	2.0	2.0	2.2
S.E.M.	0.2	0.3	0.4	0.3	0.2	0.2	0.1	0.1	0.1	0.3
CONTROLS										
MEAN	4.2	4.2	3.5	2.2	1.3	1.5	1.2	1.4	2.1	2.2
S.E.M.	0.3	0.3	0.4	0.3	0.2	0.2	0.2	0.2	0.5	0.7
Significance of difference	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS

TABLE 13.II Growth hormone concentrations (mu/l) before and after the injection of insulin (0.1 U/kg body weight) in 4 patients with Huntington's chorea and the mean concentration in 10 control subjects.

	Growth hormone mu/l									
	Time (min)									
Patients	0	0	10	20	25	30	35	40	45	60
1	4.0	4.0	4.0	12.0	12.0	96.0	72.2	80.1	84.2	64.0
2	8.0	8.2	8.0	9.0	16.0	84.0	80.1	78.1	74.1	80.0
3	11.2	12.6	10.0	11.6	12.0	46.1	48.4	60.2	65.3	78.2
4	8.0	8.4	8.0	9.0	16.0	82.4	80.0	80.0	74.3	75.4
Mean	7.8	8.3	7.5	10.4	14.0	77.1	70.2	74.6	74.5	74.4
S.E.M.	1.5	1.8	1.3	0.8	1.2	10.8	7.5	4.8	3.9	3.6
Controls										
Mean	7.8	7.8	7.8	8.0	8.0	11.0	29.4	49.4	71.4	78.6
S.E.M.	1.6	1.6	1.6	1.0	4.0	3.6	8.4	25.8	17.0	9.0
Significance of difference	NS	NS	NS	NS	NS	P .01	P .001	NS	NS	NS

DISCUSSION

Evidence for altered hGH release in patients with Huntington's chorea includes the observations that hGH is not suppressible by hyperglycaemia and that the hGH response to L-dopa is suppressible by glucose in patients but not in controls (Podolsky and Leopold, 1974). The earlier rise in hGH in the patients in the present investigation is consistent with other published reports (Keogh et al., 1976; Phillipson and Bird, 1976) and helped to confirm the diagnosis. Growth hormone responses to hypoglycaemia and hyperglycaemia are standard methods of investigating the integrity of the hypothalamic/pituitary axis and the presence of altered hGH responses in patients with Huntington's chorea strongly suggests that there may be altered hypothalamic function in this disorder.

Growth hormone release is known to be modulated by dopaminergic, noradrenergic and serotonergic mechanisms (Imura et al., 1973; Nakai et al., 1973; Lal et al., 1975; Frohman and Stachura, 1975; see also Section III). The hGH response to hypoglycaemia is blocked by phentolamine (Blackard and Heidingsfelder, 1968) and is impaired by phenothiazines (Sherman et al., 1974; Chapter 9) and cyproheptadine (Smythe and Lazarus, 1974). The earlier hGH response to hypoglycaemia in patients with Huntington's chorea might conceivably involve an alteration of catecholaminergic or serotinergic release of hGH. This might involve

an earlier release of neurotransmitter from presynaptic terminals or an increased sensitivity of postsynaptic receptors. Keogh and co-workers (1976) suggested that there may be hypersensitivity of dopamine receptors involved in hGH release as there is considerable evidence to implicate dopamine in the disorder. Alternatively, these authors suggest that the earlier response might be due to withdrawal of phenothiazines but the patients in the present investigation all had an earlier hGH response and none of them had received phenothiazine treatment.

It would be of considerable value to examine neuroendocrine responses in Huntington's chorea to determine if there is any evidence for altered catecholaminergic or serotinergic function and in particular to investigate the basis for the altered hGH responses described in this disorder. It is possible that alterations in neuroendocrine mechanisms may give valuable information about the neural dysfunction in the disorder. It was therefore decided to investigate hGH and hPRL responses to various drugs affecting catecholaminergic and serotonergic neurones and the results of these studies are presented in the following chapters.

SUMMARY

1. Growth hormone responses to insulin hypoglycaemia were investigated in four patients with chorea and a probable diagnosis of Huntington's chorea.

2. Concentrations of hGH showed an earlier elevation than controls in the four patients.

3. The presence of an earlier hGH response to hypoglycaemia was consistent with a diagnosis of Huntington's chorea in all four patients. The results suggest that the growth hormone response may be of value in helping to confirm clinical diagnosis in this disorder.

CHAPTER 14

GROWTH HORMONE AND PROLACTIN RESPONSE TO BROMOCRIPTINE IN PATIENTS WITH HUNTINGTON'S CHOREA

INTRODUCTION

The results presented in the previous chapter demonstrate that the hGH response to insulin hypoglycaemia in patients with Huntington's chorea showed an earlier elevation than controls and this result is consistent with the reports of other authors who have suggested that the earlier response may be due to increased sensitivity of dopamine receptors in the hypothalamus mediating hGH release, (Keogh et al., 1976; Phillipson and Bird, 1976). Bromocriptine is a specific dopamine receptor agonist which produces an elevation of hGH and depresses prolactin hPRL concentrations in normal subjects (del Pozo et al., 1972; Cammani et al., 1975; Chapter 7). It was therefore decided to investigate the hGH response to bromocriptine in patients with Huntington's chorea to determine if there was any evidence for altered sensitivity of dopamine receptors mediating hGH release and the results are reported in this chapter.

METHOD

Subjects

Twelve unrelated patients with chorea and a positive family history of Huntington's chorea were studied. The first group were all inpatients in Hartwood Hospital, Lanarkshire under the care of Dr. J. Bolt. They were all receiving a variety of drugs including phenothiazines. Six patients, two males and four females aged 43-53 years (mean 47 years) were studied.

The second group consisted of five outpatients and one inpatient from Hartwood Hospital, who had not received treatment with phenothiazines. The six patients comprised two males and four females aged 47-68 years (mean 59 years). Seven normal healthy controls, four males and three females aged 23-55 years (mean 44 years) were also studied. Consent was obtained after the purpose and procedure had been explained to the patients and their relatives. The control subjects all consented to take part in the investigation.

Procedure

Drug medication was stopped 72 hours before the investigation which was carried out in the morning following an overnight fast. The half life of hGH is relatively short (20-30 min) and it was therefore decided to take blood samples at 10 min. intervals during the period of the hGH response. Blood samples (10ml) were taken from a cannula in an antecubital vein before and at 30, 60, 90, 120, 130, 140, 150, 160, 170, 180 and 210 minutes after an oral dose of bromocriptine (2.5 mg). The plasma samples were analysed for hGH, hPRL and FFA. Significance of difference was examined with the Mann-Whitney non-parametric U-test for small samples (Mann and Whitney, 1947).

RESULTS

Details of the recent medication of the six inpatients who had received phenothiazines prior to the investigation is given in Table 14.I.

Plasma growth hormone (hGH) (Table 14.II)

Patients treated with phenothiazines.

There was no significant difference between the basal hGH concentrations of the patients and controls before taking bromocriptine. All the controls showed an increase in hGH concentrations after bromocriptine but the response of the patients was significantly lower than the controls at 160, 170, 180 and 210 minutes. Four of the patients showed an increase in hGH concentrations after bromocriptine but only three showed a peak hGH response greater than 10 mu/l and the remaining two patients failed to show a response.

Patients not treated with phenothiazines.

There was no significant difference in the resting hGH concentrations before bromocriptine between the patients and controls. Only three of the patients showed a rise in hGH concentrations after taking bromocriptine but only one had a peak greater than 10 mu/l. The hGH concentrations of the patients were significantly lower than the corresponding values for the controls at 150, 160, 170, 180 and 210 minutes.

TABLE 14.I Details of six patients with Huntington's chorea and drug medication received during treatment

Patient	Sex	Age (years)	Medication
D.G.	M	53	Chlorpromazine Benzhexol
M.N.	M	43	Chlorpromazine Orphenadrine
A.R.	F	47	Trifluoperazine Thioridazine
A. McL.	F	45	Diazepam, Fluphenazine Benzhexol Nitrazepam
A. McK	M	44	Trifluoperazine, Codeine Dichloralphenazone
M.B.	F	@¾	Chlorpromazine Thiopropazate

Plasma prolactin (hPRL) (Table 14.III)

Patients treated with phenothiazines.

Basal hPRL concentrations were markedly elevated ($>$ 1000 mu/l) in the two patients who failed to show an hGH response and raised (mean 835 mu/l) in another patient. Basal hPRL concentrations in the remaining four patients were within the range of the corresponding values for the controls (60-440 mu/l). Both the patients and the control subjects showed a consistent fall from basal hPRL concentrations after bromocriptine.

Patients not treated with phenothiazines.

There was no significant difference in hPRL concentrations after taking bromocriptine. Four of the five patients showed a consistent fall from resting hPRL concentrations after taking bromocriptine.

Plasma free fatty acids (Fig 14.I)

Concentrations of FFA were significantly greater in the patients not treated with phenothiazines ($P < 0.02$ all samples). There was no significant difference in FFA concentrations between patients treated with phenothiazines and controls. The administration of bromocriptine was not associated with any significant change in FFA concentrations in either group of patients or the controls.

TABLE 14.II Plasma growth hormone concentrations (mu/l) before and after the administration of bromocriptine (2.5 mg) in twelve patients with Huntington's chorea (six patients receiving phenothiazines and six patients not receiving phenothiazines) and in seven normal control subjects.

TABLE 14.II

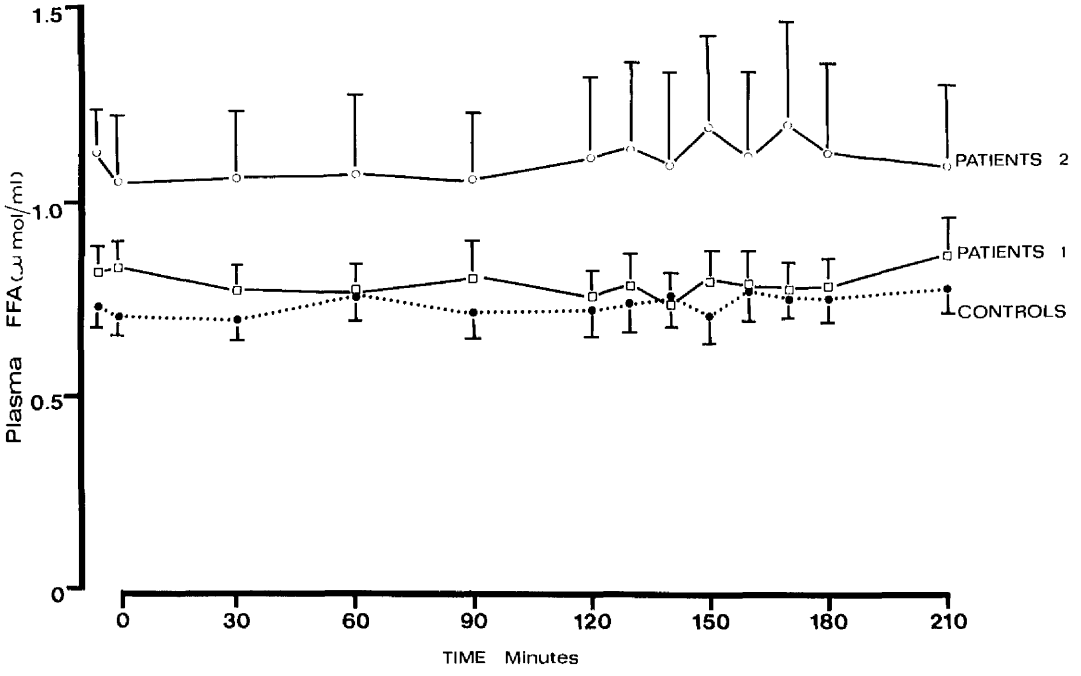
Patients Unit No.	Time Minutes	0	30	60	90	120	130	140	150	160	170	180	210
I Phenothiazine treated													
016665		2.0	3.0	3.0	2.8	4.0	4.0	7.0	10.0	15.0	-	-	-
012367		1.6	2.4	1.4	1.2	2.6	4.8	4.2	3.8	3.8	6.0	6.0	6.2
016867		16.4	10.0	4.8	9.6	10.6	20.4	27.0	24.4	13.4	12.0	12.0	7.8
011267		1.6	1.4	1.0	1.4	1.0	0.4	0.6	1.0	1.4	2.8	2.6	3.2
016389		1.0	1.0	0.6	0.4	3.8	7.8	10.0	12.8	10.4	9.0	6.6	4.0
011377		2.4	1.2	1.0	1.0	0.8	0.8	1.2	0.8	2.0	1.4	3.6	1.6
	Mean	4.2	3.2	2.0	2.7	3.8	6.4	8.3	8.8	7.7	6.2	6.2	4.6
	SEM ±	2.5	1.4	0.7	1.4	1.5	3.0	4.0	3.7	2.5	2.0	1.6	1.1
II Non-phenothiazine treated													
013511		1.2	0.6	0.4	0.4	14.0	14.0	14.0	1.2	10.4	4.2	6.6	8.0
808794		1.8	0.8	0.8	4.0	2.6	2.4	1.8	1.4	1.2	1.0	1.0	0.8
806513		6.2	2.8	3.6	6.8	5.2	7.4	6.4	7.4	9.0	5.0	5.8	7.0
227498		7.0	3.4	3.4	3.0	3.4	2.6	2.4	2.4	2.4	2.6	3.2	3.2
244298		0.4	0.2	0.6	3.0	7.0	6.2	4.0	2.4	2.4	2.2	2.2	2.2
823307		1.0	1.2	0.6	0.6	7.0	9.0	7.4	5.2	5.4	6.0	3.4	4.4
	Mean	2,9	1.5	1.6	3.0	6,5	6.9	6,0	3.3	5.1	3.5	3.7	4.3
	SEM ±	1.2	0.5	0.6	1.0	1.7	1.8	1.8	1.0	1.6	0.8	0.9	1.1
Controls	Mean	6.1	6.6	4.7	4.4	7.7	9.5	12.0	14.0	15.2	17.4	15.1	12.1
	SEM ±	3.0	3.2	1.8	2.7	3.0	3.7	3.4	3.1	2.7	2.1	2.4	4.9
Significance of difference between patients & controls	I	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	P<0.05	P<0.005	P<0.01	P<0.05
	II	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	P<0.01	P<0.01	P<0.002	P<0.001	P<0.05

TABLE 14.III Plasma prolactin concentrations (mu/l) before and after administration of bromocriptine (2.5 mg) in twelve patients with Huntington's chorea (six patients receiving phenothiazine drugs and six patients not receiving phenothiazines) and in seven normal control subjects.

TABLE 14.III

Patients Unit No.	Time Minutes	0	30	60	90	120	130	140	150	160	170	180	210
I Phenothiazine treated													
016665		900	840	770	650	440	600	240	300	280	–	–	–
0 12367		280	230	280	220	220	160	160	100	100	70	70	120
016867		180	200	70	36	40	60	80	80	60	40	40	40
011267		1800	1800	1800	1640	1580	1380	1240	1160	1240	1000	1000	1000
016389		60	80	80	40	40	40	60	20	60	80	80	80
011377		1020	820	640	760	600	400	420	500	400	370	320	260
	Mean	707	662	607	558	487	440	367	360	357	312	302	300
	SEM ±	271	264	266	250	237	208	183	176	185	182	406	179
II Non-phenothiaze treated													
013511		310	180	200	160	310	310	310	260	180	200	200	170
808794		120	160	120	140	140	120	100	140	100	140	80	100
806513		220	200	200	180	180	160	160	180	160	160	160	120
227498		240	200	160	120	120	160	120	120	120	160	100	100
244298		200	200	120	200	380	340	320	240	260	260	240	200
823307		360	320	260	200	200	180	180	180	160	160	180	160
	Mean	242	210	177	167	222	212	198	187	163	180	160	142
	SEM ±	35	23	22	13	42	37	39	22	23	18	25	17
Controls	Mean	184	161	161	142	140	120	121	168	140	145	141	142
	SEM ±	48	38	46	37	40	32	34	60	53	49	49	40
Significance of difference between patients & controls	I	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.
	II	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.

FIG 14.I Plasma FFA concentrations (μmol/ml, mean ± S.E.M.) before and after oral administration of bromocriptine (2.5 mg) in twelve patients with Huntington's chorea (six patients receiving phenothiazine drugs (1, □——□) and six patients not receiving phenothiazines (2, ○——○) and in seven normal control subjects (◍------●).


1.5
1.0
0.5
0
Plasma FFA (μ mol/ml)
PATIENTS 2
PATIENTS 1
CONTROLS
0
30
60
90
120
150
180
210
TIME Minutes

DISCUSSION

The development of the hyperkinetic condition characteristic of Huntington's chorea may be related to a change in the inter-relationship between dopamine and GABA-containing neurones (Bird and Iversen, 1974). Although dopamine levels are normal dopamine receptors may be hypersensitive (Barbeau, 1961, 1973; Bird et al., 1976). If there is hypersensitivity of dopamine receptors we might expect to find increased responsiveness of dopamine receptors mediating hGH release and this might be the basis for the earlier release of hGH reported in Huntington's chorea (Keogh et al., 1976; Phillipson and Bird, 1976).

There was no evidence of a significant elevation of basal hGH levels in the patients in the present study as has been reported by other investigators (Phillipson and Bird, 1976). The control subjects all showed a rise in hGH concentrations after taking bromocriptine and the results are consistent with other published findings (Tolis et al., 1975; Cammani et al., 1975). In the patients with Huntington's chorea the majority showed a reduced hGH response compared with controls. However, Parkes, Marsden, Donaldson, Galea-Debono, Walters, Kennedy and Asselman (1976) have reported that they failed to find an hGH response to 2.5 mg of bromocriptine in patients with Parkinson's disease although they did find a response to higher doses. It is possible that the dose of bromocriptine used in the present

study was too low but it was decided not to increase the dose as higher doses are known to increase chorea (Kartzinel, 1976). A further possibility is that the lower peak hGH response to bromocriptine found in the patients with Huntington's chorea is due to an alteration of growth hormone release mechanisms. The finding of a lower peak hGH response to bromocriptine suggests that the earlier hGH response reported to follow insulin induced hypoglycaemia in patients with the disorder is not due to increased sensitivity of dopamine receptors mediating hGH release.

In the investigation of patients who had previously received phenothiazines the failure to detect a consistent hGH response may have been because only 72 hours had been allowed between withdrawal of drug treatment and the investigation. Phenothiazines are known to block dopamine receptors (Anden et al., 1964) to impair hGH responses (Sherman et al., 1971; chapter 9) and to elevate hPRL concentrations (Beumont et al., 1974; Meltzer et al., 1976; chapters 8 and 9) and this may explain why the two patients with the greatest elevation of basal hPRL concentrations failed to show an hGH response. Klawans (1970) has proposed that dopamine receptor hypersensitivity may follow withdrawal of phenothiazine treatment but there was no evidence of a hypersensitive hGH response after withdrawal of phenothiazine treatment in the present study. In

the investigation of patients who had not received phenothiazines most of the patients showed a depression of hPRL concentrations after taking bromocriptine.

In the present investigation FFA concentrations were significantly greater in patients not treated with phenothiazines compared to controls. Raised concentrations of FFA (Phillipson and Bird, 1977) and hGH (Phillipson and Bird, 1976) have been reported in patients with Huntington's chorea. Growth hormone is known to promote lipolysis (Raben and Hollenberg, 1959) and it has been suggested that the elevated FFA levels found in the disorder may be due to the lipolytic action of hGH (Phillipson and Bird, 1977). However, basal FFA concentrations were elevated in the patients in the present study but there was no evidence of raised basal hGH concentrations. It is possible that the elevated FFA levels found in patients with the disorder may be due to non-specific stress factors or anxiety and that this effect is reduced in patients treated with phenothiazine tranquillisers. Further investigation of circulating catecholamine and cortisol levels would be value in investigating this possibility.

The results presented in this chapter demonstrate that the hGH response to bromocriptine is significantly reduced in patients with Huntington's chorea and suggest that there may be

an alteration of dopaminergic neurones mediating hGH release. The lower peak hGH response may be related to decreased sensitivity of dopamine receptors or to reduced levels of dopamine at the post synaptic receptor as the action of bromocriptine is dependent on intact catecholamine synthesis and storage mechanisms (Johnson, Loew and Vigouret, 1976). These suggestions merit further investigation as neuroendocrine studies may give useful information about hypothalamic neurone function in Huntington's chorea.

SUMMARY

1. Growth hormone (hGH) and prolactin (HPRL) responses to oral bromocriptine were studied in two groups of patients with Huntington's chorea and in seven healthy control subjects. The patients included six patients who had previously been treated with phenothiazines and six patients who had not received phenothiazine treatment. All medication was stopped 72 hours before the investigation which involved taking blood samples for up to 210 minutes after taking bromocriptine (2.5 mg).

2. There was no significant difference in basal hGH concentrations between the patients and controls. The hGH response to bromocriptine varied in the individual patients but the concentrations were significantly lower in the patients compared with the controls between 160 and 210 minutes. The basal concentrations of hPRL were also not different apart from the findings of elevated hPRL concentrations in three patients previously treated with phenothiazines.

3. The patients and controls showed a consistent fall in hPRL concentrations after taking bromocriptine. The lower peak hGH response to bromocriptine found in the patients suggests that there may be an alteration of dopaminergic neurones mediating hGH release.

4. Concentrations of FFA were significantly elevated in patients not treated with phenothiazines and were not related to elevated hGH concentrations.

CHAPTER 15

THE EFFECT OF CLONIDINE AND 5-HYDROXYTRYPTOPHAN ON GROWTH HORMONE AND PROLACTIN RELEASE IN HUNTINGTON'S CHOREA

INTRODUCTION

There is evidence that hGH responses are altered in patients with Huntington's chorea. This evidence includes the observation that the hGH response to insulin induced hypoglycaemia is earlier in patients with the disorder than in controls (Keogh et al., 1976; Phillipson and Bird, 1976; chapter 13). Growth hormone release is known to be modulated by hypothalamic dopaminergic, noradrenergic and serotonergic mechanisms (chapters 7, 10 and 11). In the previous chapter dopaminergic regulation of hGH release was investigated in patients with Huntington's chorea and the results did not suggest that there was an earlier hGH response to bromocriptine. It is possible that the earlier hGH response reported to follow insulin hypoglycaemia in patients with the disorder may be due to an alteration of noradrenergic or serotonergic release mechanisms and it was therefore decided to investigate hGH and hPRL responses to intravenous administration of clonidine and 5-hydroxy-L-tryptophan in patients with Huntington's chorea.

I. EFFECT OF CLONIDINE

METHOD

Patients and subjects

Four patients with chorea and a positive family history of Huntington's chorea were investigated. The patients, (2 males, 2 females) mean age 54 years (range 45-68 years) were investigated in the morning following an overnight fast. All the patients had stopped taking any medication for 72 hours before the investigation and none of the patients had recently received phenothiazine drugs. In addition, six normal healthy male subjects aged 23-35 years (mean 31 years) were studied as controls.

Procedure

Blood samples were taken from a cannula in an antecubital vein before and at 10, 20, 30, 40, 50, 60, 90 and 120 minutes after the injection of 0.1 mg clonidine HCl. (Catapress, Boehringer Ingelheim Ltd) in 20 ml of saline over 10 minutes.

Plasma samples were analysed for hGH, hPRL and FFA. Significance of difference was examined using the Mann-Whitney non-parametric U-test for small samples (Mann and Whitney, 1947).

RESULTS

Plasma growth hormone (Fig. 15.I)

There was no difference in basal hGH concentrations between patients and controls. Growth hormone concentrations increased after the injection of clonidine in the controls and concentrations at 20, 30, 40, 50 and 60 minutes were significantly greater than basal concentrations ($P<0.002$). The patients did not show any significant change from basal concentrations of hGH after the administration of clonidine and hGH concentrations in the patients were significantly lower than the corresponding values for the controls at 20, 30, 40 50 and 60 minutes ($P<0.05$, 0.02, 0.01, 0.02, 0.05 respectively).

Plasma prolactin (Fig. 15.I)

Basal hPRL concentrations were similar in the patients and controls. The concentration of hPRL in the controls at 50, 60, 90 and 120 minutes were significantly lower than the pre-injection concentrations ($P<0.05$). The patients showed a similar depression in hPRL concentrations after the injection but the difference was not significant. There was no significant difference between hPRL concentrations of patients and controls at any time during the investigation.

Plasma FFA (Fig. 15.II)

Basal pre-injection concentrations of FFA in the patients were significantly greater than controls ($P<0.01$). After the injection of clonidine there was no significant change in FFA concentrations in the controls whereas in the patients FFA concentrations decreased and concentrations at 20, 30, 40, 50, 60 and 90 minutes were significantly lower than pre-injection concentrations ($P<0.02$).

FIG. 15.I Plasma hGH and hPRL concentrations (mu/l, mean $\pm$ S.E.M.) before and after the administration of clonidine in four patients with Huntington's chorea (⊙——⊙) and in six normal control subjects (○----○).

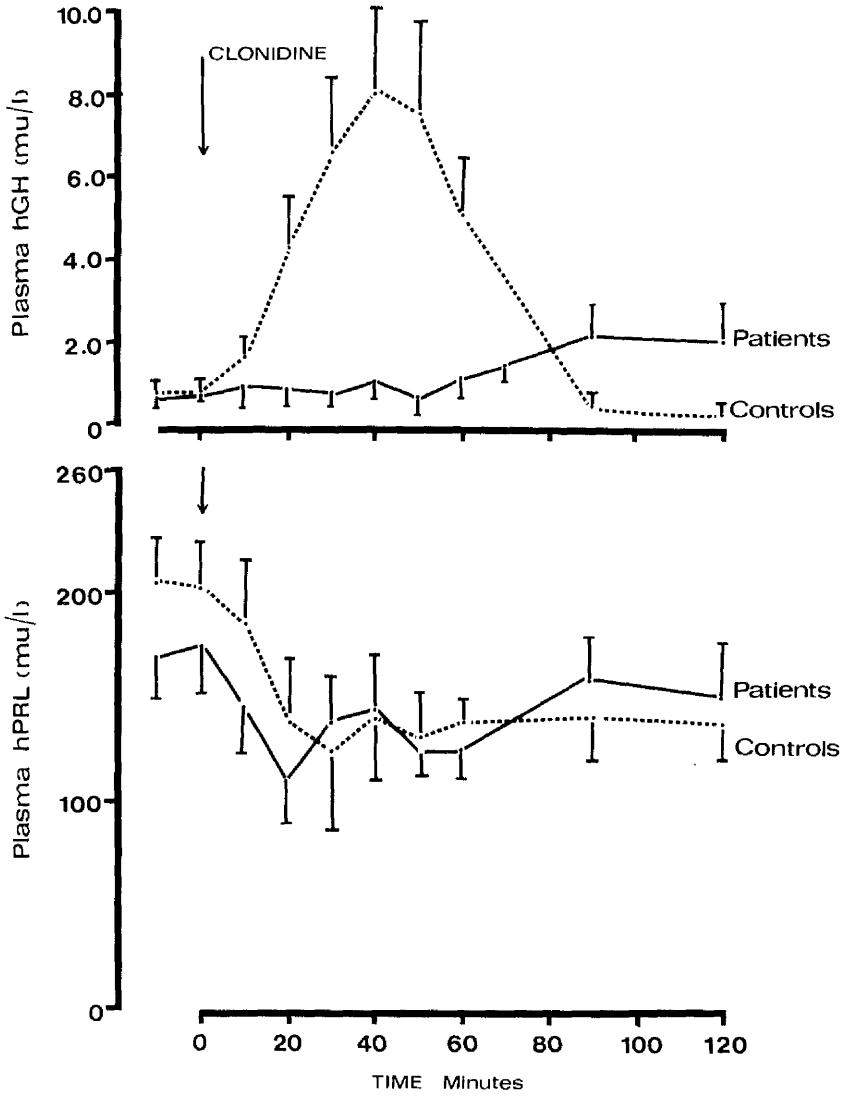
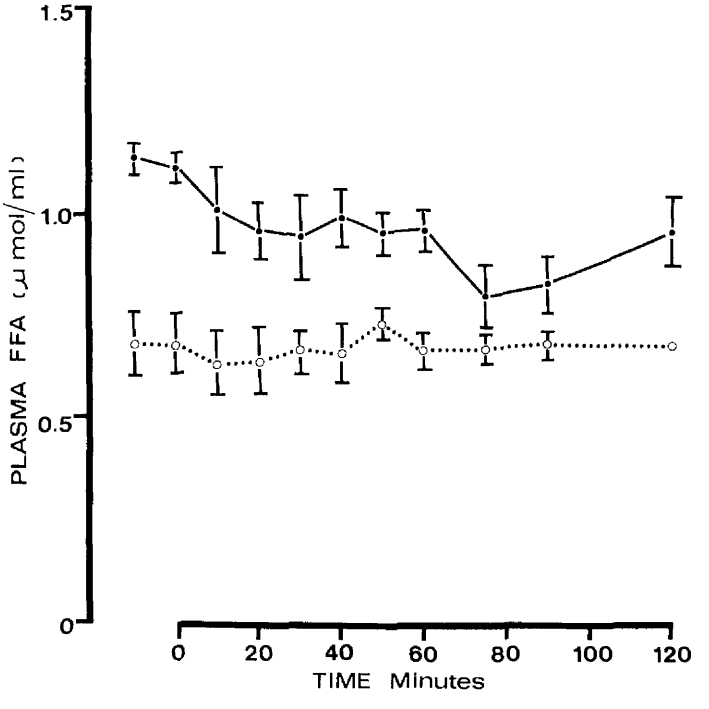

10.0
8.0
6.0
4.0
2.0
0
CLONIDINE
Plasma hGH (mu/l)
Patients
Controls
260
200
100
0
Plasma hPRL (mu/l)
Patients
Controls
0
20
40
60
80
100
120
TIME Minutes

FIG. 15.II Plasma free fatty acids (μmol/ml, mean ± S.E.M.) before and after the administration of clonidine in four patients with Huntington's chorea (●——●) and in six normal control subjects (○----○).


1.5
1.0
0.5
0
PLASMA FFA (μ mol/ml)
0
20
40
60
80
100
120
TIME Minutes

II. THE EFFECT OF 5-HYDROXY-L-TRYPTOPHAN

METHOD

Patients and subjects

Six patients with chorea and a positive family history of Huntington's chorea were investigated. The patients, (2 males, 4 females) aged 47-67 years (mean age 55 years) were all inpatients at Hartwood Hospital, Lanarkshire. They were investigated in the morning following an overnight fast and all medication was stopped for 72 hours before the investigation. In addition, five normal healthy control subjects (4 males, 1 female) mean age 34 years (range 28-40 years) were also studied.

Procedure

Blood samples were taken from a cannula in an antecubital vein before and at 10, 20, 30, 40, 50, 60, 75, 90, 105 and 120 minutes after the infusion of 20 ml of a solution containing 1mg/ml 5-hydroxy-L-tryptophan (B.D.H. Limited) over 10 minutes. Plasma samples were analysed for hGH and hPRL. Significance of difference was examined using the Mann-Whitney non-parametric U-test for small samples (Mann and Whitney, 1947).

Procedure

Blood samples were taken from a cannula in an antecubital vein before and at 10, 20, 30, 40, 50, 60, 75, 90, 105 and 120 minutes after the infusion of 20 ml of a solution containing 1 mg/ml 5-hydroxy-L-tryptophan over ten minutes. 5-hydroxyl-L-tryptophan (B.D.H. Limited) was made up in 0.9% saline and sterilized by filtration. Blood samples were analysed for glucose and plasma samples were anlaysed for hGH, hPRL and FFA. Significance of difference was examined using the Mann-Whitney non-parametric U-test for small samples (Mann and Whitney, 1947).

RESULTS

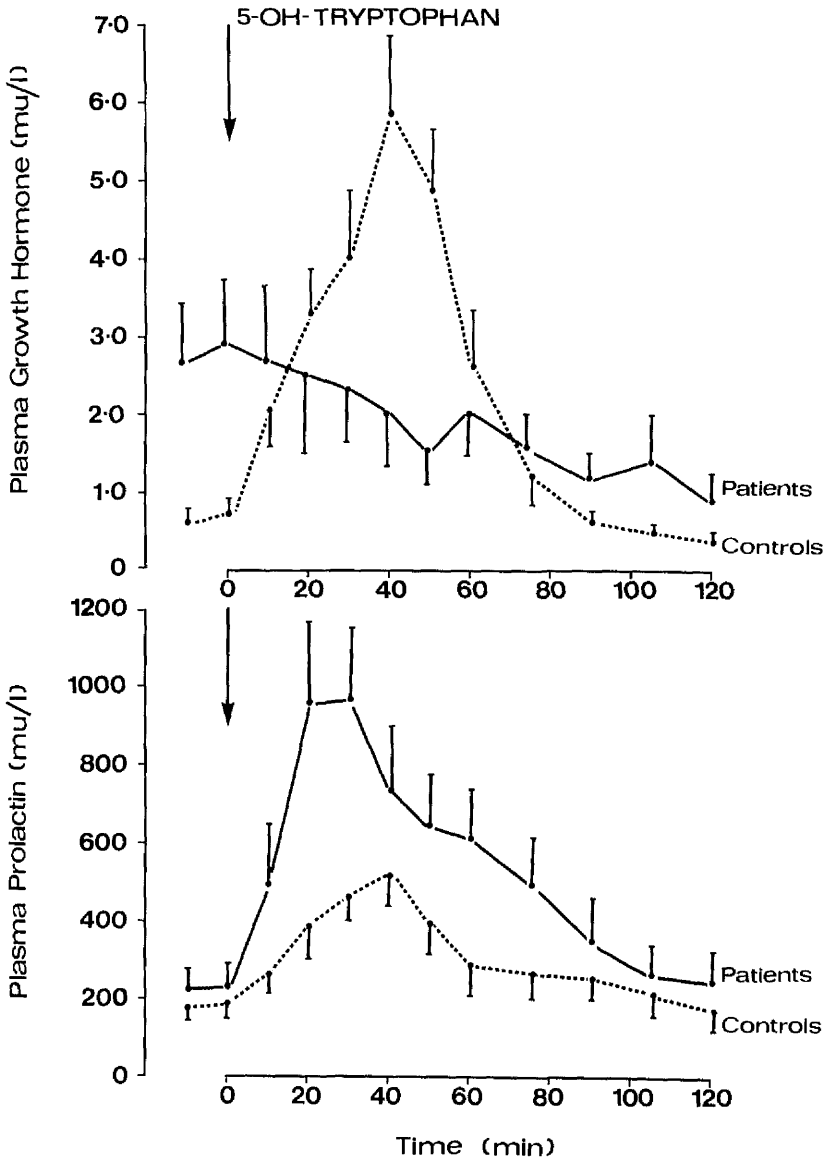
Plasma growth hormone (Fig. 15.III)

Basal hGH concentrations were significantly greater ($P<0.05$) in the patients compared to the controls. Plasma hGH concentrations increased after the administration of 5-HTP in the controls and concentration at 20, 30, 40 and 50 minutes were significantly greater than basal concentrations ($P<0.05$, 0.05, 0.005 and 0.005 respectively). There was no significant change in hGH concentrations after administration of 5-HTP in the patients and concentration in the patients were significantly lower than corresponding control concentrations at 40 and 50 minutes ($P<0.05$).

Plasma prolactin (Fig. 15.III)

Basal hPRL concentrations were similar in both patients and controls. In both groups of subjects hPRL concentrations increased after administration of 5-HTP. The concentration of hPRL in the patients reached a peak level earlier and were significantly greater than control concentrations at 30 and 40 minutes ($P<0.05$ and 0.01 respectively).

FIG. 15.III Plasma hGH and hPRL concentrations (mu/l, mean ± S.E.M.) before and after the administration of 5-hydroxy-L-tryptophan in six patients with Huntington's chorea (●——●) and in five normal control subjects (●----●).


5-OH-TRYPTOPHAN
Plasma Growth Hormone (mu/l)
7·0
6·0
5·0
4·0
3·0
2·0
1·0
0
Patients
Controls
0
20
40
60
80
100
120
Plasma Prolactin (mu/l)
1200
1000
800
600
400
200
0
Patients
Controls
0
20
40
60
80
100
120
Time (min)

DISCUSSION

Basal hPRL and hGH concentrations have been reported to be elevated in patients with Huntington's chorea (Caine, Kartzinel, Ebert and Carter, 1977; Caraceni, Panerai, Parati, Cocchi and Muller, 1977; Phillipson and Bird, 1976). In the present investigation there was no evidence of increased basal hPRL concentrations in the patients and hGH concentrations were not significantly different before the infusion of clonidine and, although there was evidence for significantly greater hGH concentrations before the administration of 5-HTP, the concentrations in the patients were within the range of basal concentrations found in normal subjects.

Growth hormone release in response to the infusion of clonidine was apparently absent in the patients in the present investigation. The patients had not received treatment with phenothiazine drugs and the absence of an hGH response was not therefore related to the blocking effects of these drugs on noradrenergic receptors (Sourkes, 1975). It is possible that the dose of clonidine administered was insufficient to produce a response in the patients but this seems unlikely as the same dose produced significant elevation of hGH in controls and produced a similar suppression of hPRL in both patients and controls. A further possibility is that there may be decreased sensitivity of hypothalamic noradrenergic receptors stimulating

hGH release. However, the present investigations on the effects of clonidine involved only four patients and further investigations will be required to confirm the present observations.

Patients with Huntington's chorea have been reported to have elevated resting FFA concentrations (Phillipson and Bird, 1977; Chapter 14). Basal FFA concentrations were raised in the patients in the present study but decreased progressively following the infusion of clonidine. Urinary catecholamine excretion has been reported to be reduced by clonidine (Hedeland, Dymling and Hokfelt, 1971) and the fall in FFA concentrations in the patients in the present investigation may be due to a decrease in circulating catecholamine concentrations as catecholamines are known to promote lipolysis (Havel and Goldfien, 1959).

Oral administration of 5-HTP (50-100 mg) in patients with the disorder is reported to produce marked exacerbation of motor symptoms (Lee, Markham and Clark, 1968). This suggests that serotonin may be involved in the production of chorea although the possibility that the effect of 5-HTP on chorea is related to release of dopamine cannot be excluded as administration of 5-HTP to animals is known to release dopamine from dopaminergic neurones (Fuxe, Butcher and Engel, 1971). The

elevation of hPRL concentrations in response to 5-HTP in the present investigation does not suggest that 5-HTP released dopamine as dopamine is known to inhibit hPRL release (Zacur et al., 1976). In the patients intravenous 5-HTP produced a greater elevation of hPRL levels but there was no significant hGH response. This finding of a greater hPRL response is not consistent with the report of a delayed rise in hPRL concentrations following intramuscular injection of chlorpromazine in patients with the disorder, (Hayden, Vinich, Paul and Beighton, 1977). Hayden and co-workers suggested that the delayed rise in hPRL may be related to increased sensitivity of dopaminergic inhibitory influences on hPRL release whereas the present observation of a greater hPRL response to 5-HTP suggests that there is increased sensitivity of serotonergic recetpors stimulating hPRL release. The absence of an hGH response to 5-HTP in the patients may have been due to previous treatment with phenothiazine drugs as these drugs are known to block serotonergic receptors (Forham and Stachura, 1975). However, an elevation of hPRL concentrations was present in all the patients and the patients had not received any medication for 72 hours prior to the investigation. In addition, phenothiazines are known to elevate hPRL concentrations (Beumont et al., 1974; Meltzer et al., 1976) and the absence of

raised hPRL concentrations in the patients before 5-HTP administration suggests that there was no blocking effect of phenothiazines on dopamine receptors 72 hours after withdrawal of medication.

The present investigations did not demonstrate any hypersensitivity of noradrenergic or serotonergic receptors stimulating hGH release in patients with Huntington's chorea. However, the presence of a greater hPRL response to 5-HTP administration does suggest that there may be enhanced responsiveness of serotonergic receptors stimulating hPRL release and this result merits further investigation of hPRL responses in patients with the disorder.

SUMMARY

1. Noradrenergic and serotonergic regulation of hGH and hPRL release was investigated in patients with Huntington's chorea by examining hGH and hPRL concentrations after the intravenous administration of clonidine (0.1 mg) and 5-hydroxy-L-tryptophan (20 mg) on two separate occasions.

2. Basal concentrations of both hGH and hPRL were similar in both the patients and controls on the two occasions.

3. Intravenous clonidine produced a significant rise in hGH and a significant fall in hPRL concentrations in controls. In the patients, the hGH response was absent although hPRL concentrations showed a similar suppression to controls. In addition, basal FFA concentrations were elevated in the patients and decreased progressively after the administration of clonidine.

4. Concentrations of both hGH and hPRL increased significantly after the intravenous administration of 5-HTP in the controls. In the patients hGH concentrations were significantly lower than controls and the rise in hPRL was greater than controls after intravenous 5-HTP.

5. The results did not demonstrate any hypersensitivity of noradrenergic or serotonergic receptors stimulating hGH release although the greater hPRL response to 5-HTP administration suggests that there may be enhanced responsiveness of serotonergic receptors stimulating hPRL release in patients with Huntington's chorea.

CHAPTER 16

GENERAL DISCUSSION AND SUGGESTIONS FOR FURTHER INVESTIGATION

The studies presented in this thesis all involve aspects of the regulation of hGH and hPRL release. The first two sections describe studies of the effects of diphenylhydantoin and alcohol and include observations on the action of these drugs on the release of anterior-pituitary hormones. In the third section hypothalamic regulation of hGH and hPRL release was investigated and methods developed for the study of neuroendocrine responses in patients.

The studies of the effects of diphenylhydantoin suggest that this drug potentiates hGH responses to exercise and the possibility that this involves an action on serotonergic hGH release mechanisms merits further investigation. The greater levels of FFA, glycerol and ketones after exercise with DPH suggest that DPH produced delayed re-esterification of FFA and the possibility that this is due to impaired insulin release requires further investigation. Studies of glucose loading demonstrated a small impairment in glucose tolerance and insulin release in epileptics receiving long term treatment with DPH This finding is not consistent with other published observations and further investigation of a larger group of patients is indicated. Basal growth hormone concentrations in the

epileptics were similar to controls and showed the normal suppression following glucose loading. These observations suggest that the enhanced hGH response to exercise in normal subjects is an effect of acute DPH treatment and that long term treatment is not associated with increased basal hGH concentrations.

The effect of alcohol on hGH and hPRL concentrations was investigated to determine if alcohol altered hypothalamic/pituitary function. Alcohol had no effect on basal hGH or hPRL concentrations although this may be due to the relatively low dose of alcohol used in these studies. However, studies during exercise did demonstrate that alcohol enhances the hGH response to exercise. Further investigation of the effect of alcohol on other hGH responses is indicated as the mechanism of this action of alcohol is unknown. Moderate doses of alcohol have been reported to have a reserpine like action in displacing catecholamines from intracellular sites in the nervous system (Ritchie, 1966). Such an action would be consistent with the observation of a greater hGH response to exercise and with the report that alcohol elevates hGH in normal subjects (Bellet et al., 1970). However, the hGH response to arginine

infusion has been reported to be impaired by alcohol (Tamburrano et al., 1976). The possibility that the different effect of alcohol on the hGH responses to exercise and arginine infusion is related to different hypothalamic regulation of these responses requires further investigation.

In an attempt to examine further the effect of alcohol on hypothalamic/pituitary function, hGH and hPRL release was examined in patients with a progressive alcohol problem. The results demonstrate that there is impaired release of hGH in response to exercise and insulin hypoglycaemia in some alcoholis and examination of the patients' recent clinical history suggested that this impairment may be related to the development of alcohol withdrawal syndrome. The recent report that the TSH response to TRH is impaired in some alcoholics during withdrawal (Loosen and Prange, 1977) is consistent with the suggestion that there may be a general impairment of release of pituitary hormones in alcohol withdrawal although further investigation is required. The possibility that impaired release of pituitary hormones during alcohol withdrawal is related to an alteration of hypothalamic regulating mechanisms merits further investigation as such studies may have important implications for the understanding of alcohol

dependance and the treatment of alcoholism.

The suggestion that both DPH and alcohol exert an effect on hypothalamic/pituitary function could be further investigated using the various methods described in the studies presented in Section III of this thesis. These studies add further support to the evidence suggesting that hGH and hPRL release is influenced by hypothalamic dopaminergic, noradrenergic and serotonergic receptors. Studies with the dopamine receptor agonist, bromocriptine, demonstrated an elevation of hGH in normal subjects and a suppression of hGH and hPRL concentrations in acromegaly. The results confirm other published findings and support the suggestion that bromocriptine may be of value in the treatment of acromegaly and in the investigation of dopaminergic regulation of hGH and hPRL release.

Studies of the effects of the phenothiazine derivative, fluphenazine, demonstrate that this drug elevates hPRL concentrations, enhances the hPRL response to hypoglycaemia and impairs the hGH response to this stimulus. The presence of increased basal hPRL after treatment with fluphenazine suggests that this drug blocks dopaminergic inhibitory influences on hPRL release. The impaired hGH response may

be due, at least in part, to the blockade of dopaminergic receptors but the contribution to this impairment by blockade of noradrenergic and serotonergic receptors requires further investigation. The marked variation in hPRL concentrations in individual patients receiving the same dose of fluphenazine merits further investigation in relation to individual variation in the metabolism of fluphenazine and to the sensitivity of dopaminergic receptors to phenothiazines in individual patients. This latter possible source of variation may be of particular significance as patients who demonstrate the greatest increases in hPRL following phenothiazine administration may be more sensitive to the blocking effects of these drugs on dopaminergic receptors and may also be more likely to develop extrapyramidal symptoms. Further investigation of the relationship between hPRL concentrations and the development of extrapyramidal side effects would be of value in investigating this possibility.

The administration of clonidine, a noradrenaline receptor agonist, produced significant elevation of hGH and suppressed hPRL levels in normal subjects. These observations confirm the suggestion that noradrenergic receptors stimulate hGH release and extend to man the

observation made in animals that hypothalamic noradrenergic receptors inhibit hPRL release. This evidence for the involvement of noradrenergic receptors in regulating neuroendocrine responses indicates that the effects of non-specific drugs and precursors, for example, phenothiazines and L-dopa, cannot be interpreted as involving a specific action on dopaminergic receptors. Administration of 5-hydroxy-L-tryptophan produced significant elevation of both hGH and hPRL concentrations in normal subjects and demonstrate serotonergic involvement in the regulation of hGH and hPRL release.

The release or inhibition of anterior-pituitary hormones by hypothalamic catecholaminergic or serotonergic receptors may be mediated by the release of peptide trophic hormones which act on anterior-pituitary cells. This aspect of the regulation of hormone release was studied by investigating the effect of somatostatin on hGH and hPRL release in response to exercise in normal subjects. Infusion of somatostatin produced complete suppression of the hGH response to exercise confirming the growth hormone release inhibiting properties of this peptide. Somatostatin had no effect on hPRL release but did produce greater suppression of insulin concentrations during exercise and also suppressed the

glucagon response to exercise. Decreased glucose concentrations were observed during the infusion of somatostatin suggesting that the suppression of glucagon levels led to decreased hepatic glucose production. These effects on insulin, glucagon and glucose are consistent with the reports of other investigators. The suppression of the hGH response did not appear to alter FFA or glycerol concentrations during exercise suggesting that hGH does not play an important role in the mobilisation of fat during exercise. The possibility that the rebound rise in hGH concentrations after completion of the somatostatin was due to hypoglycaemia during the infusion requires further investigation.

Huntington's chorea is a degenerative disorder of the central nervous system. Neuropathological involvement of the hypothalamus has been demonstrated in this disorder (Bruyn, 1968; Bruyn, 1973) and patients have been reported to have an earlier release of hGH in response to insulin induced hypoglycaemia (Keogh et al., 1976; Phillipson and Bird, 1976). This earlier release of hGH following hypoglycaemia was confirmed in studies presented in this thesis and it appears that the presence of this altered hGH

response may be of value in the diagnosis of Huntington's chorea. In order to investigate if the earlier hGH response to hypoglycaemia was related to altered sensitivity of hypothalamic dopaminergic, noradrenergic or serotonergic receptors stimulating hGH release, the hGH and hPRL responses to bromocriptine, clonidine and 5-hydroxy-L-tryptophan were studied in patients with the disorder. Studies with bromocriptine demonstrated a significantly impaired hGH response suggesting that hypothalamic dopaminergic receptors may have reduced sensitivity or that there is impaired storage or release of dopamine from presynaptic neurones. Administration of clonidine also demonstrated a significantly impaired hGH response. However, the studies with clonidine involved only four subjects and further investigation is required to confirm these findings. Intravenous administration of 5-hydroxy-L-tryptophan failed to produce a significant hGH response in patients with the disorder although the hPRL response was significantly greater than controls. Taken together, these results do not provide any evidence of increased sensitivity of dopaminergic, noradrenergic or serotonergic receptors stimulating hGH release in patients with Huntington's chorea. The presence

of significantly reduced hGH responses to bromocriptine, clonidine and 5-HTP requires further investigation. The presence of a greater hPRL response to 5-HTP administration suggests that there is enhanced responsiveness of seroto-nergic receptors stimulating hPRL release and further investigation of hPRL release in patients with the disorder are indicated. A major problem associated the investigation of neuroendocrine responses in patients with Huntington's chorea is the widespread use of neuroleptic drugs in the treatment of this disorder. It is generally supposed that withdrawal of phenothiazine drugs leads to increased sensitivity of receptors which are affected by the blocking action of these drugs. The extent to which the sensitivity of hypothalamic receptors regulating hGH and hPRL release is altered following withdrawal of phenothiazines requires further investigation. Further studies of hypothalamic regulation of anterior-pituitary hormone release in patients with Huntington's chorea may be of value in identifying alterations of hypothalamic neural function in this disorder.

REFERENCES

ALFORD, F.P., BLOOM, S.R., NABARRO, J.D.N., HALL, R., BESSER, G.M., COY, D.H., KASTIN, A.J. and SCHALLY, A.V. (1974). Glucagon control of fasting glucose in man. Lancet, ii, 974-977.

ANDEN, N., CARLSSON, A. and HAGGENDAL, J. (1969). Adrenergic mechanisms. Annual Review of Pharmacology, 9, 119-134.

ANDEN, N.E., ROOS, B.E. and WERDINIUS, B. (1964). Effects of chlorpromazine, haloperidol and reserpine on the levels of phenolic acids in rabbit corpus striatum. Life Sciences, 3, 149-158.

ANDEN, N.E., CORRODI, H., FUXE, K., HOKFELT, B., HOKFELT, T., RYDIN, C. and SVENSSON, T. (1970). Evidence for a central noradrenaline receptor stimulation by clonidine. Life Sciences, 9, 513-523.

ANDREANI, D., TAMBURRANO, G. and JAVICOLI, M. (1976). Alcohol hypoglycaemia: hormonal changes. In: Hypoglycaemia: Proceedings of the European Symposium, Rome, Ed. D. Andreani, P. Lefebvre and V. Marks, pp 99-105. George Thieme, Stuttgart.

APOSTOLAKIS, M., KAPETANAKIS, S., LAZOS, G. and MADENA-PYRGAKI, A. (1972). Plasma prolactin activity in patients with galactorrhoea after treatment with psychotropic drugs. In: Lactogenic Hormones, Ed. G.E.W. Wolstenholme and J. Knight; pp 349-354. Churchill-Livingstone, London.

BARBEAU, A. (1961). Dopamine and basal ganglia disease. Archives of Neurology (Chic.) 4, 97-102.

BARBEAU, A. (1973). Biochemistry of Huntington's chorea. In: Advances in Neurology, Ed. A. Barbeau, T.N. Chase and G.W. Paulson, 1, 473-516. Raven Press, New York.

BAYLISS, W. M. and STARLING, E.H. (1904). The chemical regulation of the secretory process. Proceedings of the Royal Society, London, series B., 73, 310-322.

BELLET, S., ROMAN, L., De CASTRO, O.A.P. and HERRERA, M. (1970). Effect of acute ethanol intake on plasma 11-hydroxycorticosteroid levels. Metabolism, 19, 664-667.

BELLET, S., YOSHMINE, N., DeCASTRO, O.A., ROMAN, L., PARMA, S.S. and SANDBERG, H. (1971). The effect of alcohol ingestion on growth hormone levels: their relation to 11-hydroxycorticoid levels and serum FFA. Metabolism, 20, 762-769.

BELTON, N.R., ETHERIDGE, J.E. and MILLICHAP, J.G. (1965). Effects of convulsions and anticonvulsants on blood sugar in rabbits. Epilepsia, 6, 243-249.

BERGMEYER, H.U. and BERNT, E. (1963). Determination of glucose oxidase and peroxidase. In: Methods of Enzymatic Analysis, Ed. H.U. Bergmeyer, Weinheim, p.125.

BERLE, P., FINSTERWALDER, E. and APOSTOLAKIS, M. (1974). Comparative studies on the effect of human growth hormone, human prolactin and human placental lactogen on lipid metabolism. Hormone and Metabolic Research, 6, 347-350.

BERNARD, C. (1855). Levons de Physiologie Experimentale. Faites au College de France, Paris.

BEUMONT, P.J.V., GELDER, M.G. and FRIESEN, H.G. (1974). The effects of phenothiazines on endocrine function. 1. Patients with inappropriate lactation and amenorrhoea. British Journal of Psychiatry, 124, 413-419.

BIEBERDORF, F.A., CHERNICK, S.S. and SCOW, R.O. (1970). Effect of insulin and acute diabetes on plasma FFA and ketone-bodies in the fasting rat. Journal of Clinical Investigation, 49, 1685-1693.

BIRD, E.D., CHIAPPA, S.A. and FINK, G. (1976). Brain immunoreactive gonadotrophin-releasing hormone in Huntington's chorea and in non-choreic subjects. Nature, 260, 536-538.

BIRD, E.D. and IVERSEN, L.L. (1974). Huntington's chorea: post mortem measurement of glutamic acid decarboxylase, choline acetyltransferase and dopamine in basal ganglia. Brain, 97, 457-472.

BLACKARD, W.G. and HEIDINGSFELDER, S.A. (1968). Adrenergic receptor control mechanism for growth hormone secretion. Journal of Clinical Investigation, 47, 1407-1414.

BLACKARD, W.G. (1970). Stimulatory effect of exogenous catecholamines on plasma hGH concentrations in the presence of beta adrenergic blockade. Metabolism, 19, 547-552.

BLAUTH, C.I.A., SONKSEN, P.H., TOMPKINS, C.V. and BLOOM, S.R. (1977). The hypoglycaemic action of somatostatin in the anaesthetized dog. Clinical Endocrinology, 6, 17-25.

BLOOM, S.R. (1974). Hormones of the gastrointestinal tract. British Medical Bulletin 30, 62-67.

BLOOM, S.R., JOHNSON, R.H., PARK, D.M., RENNIE, M.J. and SULAIMAN, W.R. (1976). Differences in the metabolic and hormonal response to exercise between racing cyclists and untrained individuals. Journal of Physiology, 258, 1-18.

BONNYCASTLE, D.D., PAASONEN, M.K. and CIARMAN, N.J. (1956). Diphenylhydantoin and brain levels of 5-hydroxy-tryptamine. Nature, 178, 990-991.

BOTTGER, I., SCHLEIN, E.M., FALOONA, G.R., KNOCHEL, J.P. and UNGER, R.H. (1972). The effect of exercise on glucagon secretion. Journal of Clinical Endocrinology and Metabolism, 35, 117-125.

BOYD, .A.E., LEBONITZ, H.E. and PFEIFFER, J.B. (1970). Stimulation of growth hormone secretion by L-dopa. New England Journal of Medicine, 283, 1425-1429.

BRAZEAU, P., VALE, W., BURGUS, R., LING, N., BUTCHER, N., RIVIER, J. and GUILLEMIN, R. (1973). Hypothalamic polypeptide that inhibits the secretion of immunoreactive pituitary growth hormone. Science, 179, 77-79.

BROWN, G.M., SCHALCH, D.S. and REICHLIN, S. (1971). Hypothalamic mediation of growth hormone and adrenal stress response in the squirrel monkey. Endocrinology, 89, 694-703.

BROWN, G.M. and REICHLIN, S. (1972). Psychologic and neural regulation of growth hormone secretion. Psychosomatic Medicine, 34, 45-61.

BRUYN, G.W. (1968). Huntington's chorea, history, clinical and laboratory synopsis. In; Handbook of Clinical Neurology, Ed. P. J. Vinken and G. W. Bruyn, 6, 298-387. North Holland Amsterdam.

BRUYN, G.W. (1973). Neuropathological changes in Huntington's chorea. In: Advances in Neurology, Ed. A. Barbeau, T. N. Chase and G.W. Paulson, 1, 399-403. Raven Press, New York.

BUCKLER, J.M.H., BOLD, A.M., TABERNER, M. and LONDON, D.R. (1969). Modification of hormonal response to arginine by -adrenergic blockade. British Medical Journal, 3, 153-154.

BUSH, I.E. and SANDBERG, A.A. (1953). Adrenocortical hormones in human plasma. Journal of Biological Chemistry, 205, 783-793.

CAINE, E.D., KARTZINEL, R., EBERT, M.H. and CARTER, A.C. (1977). Dopaminergic regulation of prolactin release in patients with Huntington's disease and normal controls. Neurology, 27, 392.

CAMERON, D.P., BURGER, H.G. and CATT, K.J. (1969). Metabolic clearance rate of radioiodinated human growth hormone. Journal of Clinical Endocrinology and Metabolism, 35, 665-670.

CAMMANI, F., MASSARA, F., BELFORTE, L. and MOLINATTI, G.M. (1975). Changes in plasma growth hormone levels in normal and acromegalic subjects following administration of 2-Bromo-alpha-ergocryptine. Journal of Clinical Endocrinology and Metabolism, 40, 363-366.

CANDELISE, L., FAGLIONI, P., SPINNLER, H. (1973). Treatment of Huntington's chorea. New England Journal of Medicine, 289, 1201.

CARACENI, T., PANERAI, A.E., PARATI, E.A., COCCHI, D. and MULLER, E.E. (1977). Altered growth hormone and prolactin response to dopaminergic stimulation in Huntington's chorea. Journal of Clinical Endocrinology and Metabolism, 44, 870-875.

CASTLEDEN, C.M., and RICHENS, A. (1973). Chronic phenytoin therapy and carbohydrate tolerance. Lancet, ii, 966-967.

CAVAGNINI, F. and PERACCHI, M. (1971). Effect of reserpine on growth hormone response to insulin hypoglycaemia and to arginine infusion in normal subjects and hyperthyroid patients. Journal of Endocrinology, 51, 651-656.

CHALMERS, R.J. (1976). Studies of the alteration of carbohydrate, fat and brain amine metabolism in man in health and disease. M.Sc. thesis. University of Glasgow, Glasgow.

CHIDECKEL, E.W., PALMER, J., KOERKER, D.J., ENSINCK, J., DAVIDSON, M.B. and GOODNER, C.J. (1975). Somatostatin blockade of acute and chronic stimuli of the endocrine pancreas and the consequences of this blockade on glucose homeostasis. Journal of Clinical Investigation, 55, 754-762.

CHIODINI, P.G., LUIZZI, A., BOTALLA, L., OPPIZZI, G., MULLER, E.E. and SILVESTRINI, F. (1975). Stable reduction of plasma growth hormone levels during chronic administration of 2-bromo- -ergocryptine (CB-154) in acromegalic patients. Journal of Clinical Endocrinology and Metabolism, 40, 705-712.

CHRISTENSEN, S.E., PRANGE HANSEN, A., IVERSEN, J., LUNDBAEK, K., ORSKOV, H. and SEYER-HANSEN, K. (1974). Somatostatin as a tool in studies of basal carbohydrate and lipid metabolism in man; modifications of glucagon and insulin release. Scandinavian Journal of Clinical and Laboratory Investigation, 34, 321-325.

COBB, L.A. and JOHNSON, W.P. (1963). Hemodynamic relationships of anaerobic metabolism and plasma free fatty acids during prolonged strenuous exercise in trained and untrained subjects. Journal of Clinical Investigation, 42, 800-810.

COSTA, P.J., GLASER, G.H. and BONNYCASTLE, D.D. (1955). Effects of diphenylhydantoin (Dilantin) on adrenal cortical function: a study in non-epileptic human subjects. Archives Neurology, 74, 88-91.

CROWE, S.J., CUSHING, H. and HAMOND, J. (1910). Experimental hypophysectomy. John Hopkins Hospital Bulletin, 21, 127-169.

CUMMINGS, N.P., ROSENBLOOM, A.L., KOHLER, W.C. and WILDER, B.J. (1973). Plasma glucose and insulin responses to oral glucose with chronic diphenylhydantoin therapy. Pediatrics, 51, 1091-1093.

CURRY, S.H. (1971). Chlorpromazine; concentrations in plasma excretion in urine and duration of effect. Proceedings of the Royal Society of Medicine, 64, 285-289.

CURRY, S.H., MARSHAL, J.H.L., DAVIS, J.M. and JANOVSKY, D.S. (1970). Chlorpromazine plasma levels and effects. Archives of General Psychiatry, 22, 289-296.

DALTON, C. and KOWALSKI, C. (1967). Automated colorimetric determination of FFA in biological fluids. Clinical Chemistry, 13, 744-751.

DAY, J.L. (1975). The metabolic consequences of adrenergic blockade: a review. Metabolism, 24, 987-996.

DEL POZO, E., BRUN DEL RE, R., VARGA, L. and FRIESEN, H.G. (1972). The inhibition of prolactin secretion in man by CB-154. Journal of Clinical Endocrinology, 35, 768-771.

DE VANE, G.W., SILER, T.M. and YEN, S.S.C. (1974). Acute suppression of insulin and glucose levels by synthetic somatostatin in normal human subjects. Journal of Clinical Endocrinology and Metabolism, 38, 913-915.

DE WIED, D. (1967). Chlorpromazine and endocrine function. Pharmacology Reviews, 19, 251-288.

DILL, R.E. (1966). Discrepancy of adrenal responses in diphenylhydantoin treated rats. Archives Internationales Pharmacodynamie et de Therapie, 160, 363-373.

EARLE, K.H. (1973). Pathology and experimental models of Huntington's chorea. In: Advances in Neurology, 1, 340-351. A. Barbeau, T.N. Chase and G.W. Paulson (Eds.). Raven Press, New York.

EDDY, R.L., JONES, A.L., CHAKMAKJIAN, Z.H., and SILVERTHORNE, M.C. (1971). Effect of levodopa (L-dopa) on human hypophysial trophic hormone release. Journal of Clinical Endocrinology and Metabolism, 33, 709-712.

ETTIGI, P., LAL, S., MARTIN, J.B. and FRIESEN, H.G. (1975). Effect of sex, oral contraceptives and glucose loading on apomorphine-induced growth hormone secretion. Journal of Clinical Endocrinology and Metabolism, 40, 1094-1098.

EVERETT, J.W. (1966). The control of the secretion of prolactin. In: The Pituitary Gland, p166. G.W. Harris and B.T. Donovan (Eds.). Butterworths, London.

FARIS, B.L. and LUTCHER, C.L. (1971). Diphenylhydantoin-induced hyperglycaemia and impaired insulin release: Effect of dosage. Diabetes, 20, 177-181.

FERGUSTROM, J.P. and WURTMAN, R.J. (1971). Brain serotonin content: Physiological dependence on plasma tryptophan levels. Science, 173, 149-152.

FISHER, C., INGRAM, W.R. and RANSON, S.W. (1938). In: Diabetes insipidus and the neuro-hormonal control of water balance, p 212. Edwards Bros. (Ed). Ann. Arbor, Michigan.

FORBES, A.P., HENNEMAN, P.H., GRISWOLD, G.C. and ALBRIGHT, F.C. (1954). Syndrome characterized by galactorrhea, amenorrhea and low urinary FSH: comparison with acromegaly and normal lactation. Journal of Clinical Endocrinology, 14, 265-271.

FRANKS, S., JACOBS, H.S. and NABARRO, J.D.N. (1976). Prolactin concentrations in patients with acromegaly: clinical significance of response to surgery. Clinical Endocrinology, 5. 63-72.

FRANTZ, A.G., KLEINBERG, D.L. and NOEL, G.L. (1972). Physiological and pathological secretion of human prolactin studied by in vitro bioassay. In: Lactogenic Hormones, 137-150. G.E.W. Wolstenholme, and J. Knight (Eds.). Churchill Livingstone, Edinburgh and London.

FRIESEN, H.G., GUYDA, H., HUANG, P., TYSON, J.E. and BARBEAU, A. (1972). Functional evaluation of prolactin secretion: a guide to therapy. Journal of Clinical Investigation, 51, 706-709.

FROHMAN, L.A. and STACHURRA, M.E. (1975). Neuropharmacological control of neuroendocrine function in man. Metabolism 4, 211-234.

FUXE, K., BUTCHER, L.L. and ENGEL, J. (1971). DL-5-hydroxytryptophan-induced changes in central monoamine neurones after peripheral decarboxylase inhibition. Journal of Pharmacy and Pharmacology, 23, 420-424.

FUXE, K. and HOKFELT, T. (1969). In: Frontiers in Neuroendocrinology, p47. W.F. Ganong and L. Martini (Eds.). Oxford University Press, London.

GADE, E.B. and HEINRICH, K. (1955). Klinische Boebachtungen bei Megaphenbehandlung in der Psychiatrie. Nervenarzt, 26, 49-54.

GERICH, J.E., LORENZI, M., SCHNEIDER, V. and FORSHAM, P.H. (1974). Effect of somatostatin on plasma glucose and insulin responses to glucagon and tolbutamide in man. Journal of Clinical Endocrinology and Metabolism, 39, 1057-1060.

GLICK, S. and GOLDSMITH, S. (1968). The physiology of growth hormone secretion In: Growth Hormone, p 84. A. Pecile and E.E. Muller (Ed.). Excerpta Medica Foundation, New York.

GOLDBERG, E.M. and SANBAR, S.S. (1969). Hyperglycaemic, non-ketotic coma following administration of Dilantin (diphenylhydantoin). Diabetes, 18, 101-106.

GORDON, G.G. and SOUTHERN, A.L. (1977). Metabolic effects of alochol on the endocrine system. In: Metabolic Effects of Alcoholism. C.S. Lieber (Ed.). P.249-301. MTP Press Limited.

GOUDIE, J.H. and BURNETT, D. (1973). A gas chromatographic method for simultaneous determination of phenobarbitone, primidone and phenytoin in serum using a nitrogen detector. Clinica Chemica Acta, 43, 423-429.

GREENWOOD, F.C. and LANDON, J. (1966). Growth hormone secretion in response to stress in man. Nature, 210, 540-541.

GREER, M.A. (1957). Studies of the influence of the central nervous system on anterior pituitary function. Recent Progress in Hormone Research, 13, 67-104.

GUILLEMIN, R. (1964). Hypothalamic factors releasing pituitary hormones. Recent Progress in Hormone Research, 20, 89-122.

GUYDA, H.J. and FRIESEN, H.G. (1971). The separation of monkey prolactin from monkey growth hormone by affinity chromatography. Biochemistry and Biophysics Research Communications, 42, 1068-1075.

HALL, R., BESSER, G.M., SCHALLY, A.V., COY, D.H., EVERED, D., GOLDIE, D.J., KASTIN, A.J., McNEILLY, A.S., MORTIMER, C.H., PHENEKOS, C., TURNBRIDGE, W.M.G. and WEIGHTMAN, D. (1973). Action of growth hormone release inhibiting hormone in healthy men and acromegaly. Lancet, ii, 581-584.

HANSEN, A.P. (1971). The effect of adrenergic blockade on the exercise induced serum growth hormone rise in normals and juvenile diabetics. Journal of Clinical Endocrinology and Metabolism, 33, 807-812.

HANSEN, A.P., ORSKOV, H., SEYER-HANSEN, K. and LUNDBAEK, K. (1973). Some actions of growth hormone release inhibiting factor. British Medical Journal, III, 523-524.

HARRIS, G.W. (1955). In: Neural control of the pituitary Gland. Edward Arnold, London.

HARRIS, G.W. and GREEN, J.D. (1947). The neurovascular link between the neurophypophysis and the adenohypophysis. Journal of Endocrinology, 5, 136-146.

HARTLEY, L.H., MASON, J.W., HOGAN, R.P., JONES, L.G., KOTCHEN, T.A., MONGEY, E.H., WHERRY, F.E., PENNINGTON, L.L. and RICKETTS, P.T. (1972). Multiple hormonal responses to prolonged exercise in relation to physical training. Journal of Applied Physiology, 33, 607-610.

HARTOG, M., HAVEL, R.J., COPINSCHI, G., EARLL, J.M. and RITCHIE, R. (1967). The relationship between changes in serum levels of growth hormone and mobilization of fat during exercise in man. Quarterly Journal of Experimental Physiology, 52, 86-96.

HAVEL, R.J., and GOLDFIEN, A. (1959). The role of the sympathetic nervous system in the metabolism of free fatty acids. Journal of Lipid Research, 1, 102-108.

HAWKINS, R.D. and KALANT, H. (1972). The metabolism of ethanol and its metabolic effects. Pharmacological Review, 24, 67-157.

HAYDEN, M.R., VINIK, A.I., PAUL, M. and BEIGHTON, P. (1977). Impaired prolactin release in Huntington's chorea. Lancet, ii, 423-426.

HEDELAND, H., DYMLING, J.F. and HOKFELT, B. (1971). Pharmacological inhibition of adrenaline secretion following insulin induced hypoglycaemia in man: the effect of catapressan. Acta Endocrinologica, 67, 97-103.

HERMANSEN, L., PRUETT, E.D., OSNES, J.B. and GIERE, F.A. (1970). Blood glucose and plasma insulin in response to maximal exercise and glucose infusion. Journal of Applied Physiology, 29, 13-16.

HOHORST, H.J., KREUTZ, F.H., and BUCHER, T. (1959). Uber metabolitgehalte und metabolitkonzentrationen in der leber der ratte. Biochemische Zeitschrift, 332, 18-46.

HOLMGREN, A. and STROM, G. (1959). Blood lactate concentration in relation to absolute and relative work load in normal men, and in mitral stenosis, atrial septal defect and vasoregulatory asthenia. Acta Medica Scandinavica, 163, 185-193.

HORE, B.D. (1977). Clinical features and treatment of alcoholism. British Journal of Hospital Medicine, 18, 106-116.

HUNTER, W.M., FONSEKA, C.C. and PASSMORE, R. (1965a). The role of growth hormone in the mobilization of fuel for muscular exercise. Quarterly Journal of Experimental Physiology, 50, 406-416.

HUNTER, W.M., FONSEKA, C.C. and PASSMORE, R. (1965b). Growth hormone, important role in muscular exercise in adults. Science, 150, 1051-1052.

HUNTER, W.M. and GANGULI, P.G. (1971). The separation of antibody bound from free antigen. In Radioimmunoassay Methods, pp 243-257. K.E. Kirkham and W.M. Hunter (Eds.). Churchill-Livingstone, Edinburgh

IMURA, H., NAKAI, Y. and YOSHIMI, T. (1973). Effect of 5-hyroxytryptophan on growth hormone and ACTH in man. Journal of Clinical Endocrinology and Metabolism, 36, 204-206.

IRIE, M., SAKUMA, M., TSUSHIMA, T., SHIZUME, K. and NAKARO, K. (1967). Effect of nicotinic acid administration on plasma growth hormone concentrations. Proceedings of the Society for Experimental Biology and Medicine, 126, 708-711.

ITAYA, K. and UT, M. (1965). Colorimetric determination of free fatty acids in biological fluids. Journal of Lipid Research, 6, 16-20.

JACOBS, L.S., SNYDER, P.J., WILBER, J.F., UTIGER, R.O. and DAUGHADAY, W.H. (1971). Increased serum prolactin after administration of synthetic thyrotrophin releasing hormone (TRH) in man. Journal of Clinical Endocrinology and Metabolism, 33, 996-998.

JENKINS, J.S. and CONNOLLY, J. (1968). Adrenocortical response to ethanol in man. British Medical Journal, 2, 804-805.

JENNETT, S., JOHNSON, R.H. and RENNIE, M.J. (1972). Ketosis in untrained subjects and racing cyclists after strenuous exercise. Journal of Physiology, 225, 47-48P.

JOHNSON, A.M., LOEW, D.M. and VIGOURET, J.M. (1976). Stimulant properties of bromocriptine on central dopamine receptors in comparison to apomorphine, (+) - amphetamine and L-dopa.

JOHNSSON, G., FUXE, K. and HOKFELT, T. (1972). On the catecholamine innervation of the hypothalamus with special reference to the median eminence. Brain Research, 40, 172-281.

JOHNSON, R.H., PARK, D.M., RENNIE, M.J. and SULAIMAN, W.R. (1974). Hormonal responses to exercise in racing cyclists. Journal of Physiology, 241, 23P.

JOHNSON, R.H., RENNIE, M.J., WALTON, J.L. and WEBSTER, M.H.C. (1971). The effect of moderate exercise on blood metabolites in patients with hypopituitarism. Clinical Science, 40, 127-136.

JOHNSON, R.H. and WALTON, J.L. (1972). The effect of exercise upon acetoacetate metabolism in athletes and non-athletes. Quarterly Journal of Experimental Physiology, 57, 73-79.

JOHNSON, R.H., WALTON, J.L., KREBS, H.A. and WILLIAMSON, D.H. (1969). Metabolic fuels during and after severe exercise in athletes and non-athletes. Lancet, 2, 452-455.

JUCHEMS, R. and KUMPER, E. (1968). Blood lactate response to exercise. New England Journal of Medicine, 278, 912-913.

KAMBERI, I.A. (1973). The role of brain monamines and pineal indoles in the secretion of ganadotrophins and ganadotrophin releasing factors. Progress in Brain Research, 39, 261-280.

KANSAL, P.C., BUSE, J. and TALBERT, O.R. (1972). The effect of L-dopa on plasma growth hormone, insulin and thyroxine. Journal of Clinical Endocrinology and Metabolism, 34, 99-105.

KARTZIEL, R., HUNT, R.D. and CALNE, D.B. (1976). Bromocriptine in Huntington's chorea. Archives of Neurology, 33, 517-518.

KATO, Y., NAKAI, Y., IMURA, H., CHIHARA, K. and OHGO, S. (1973). Effect of 5-hyroxytryptophan (5-HTP) on plasma prolactin levels in man. Journal of Clinical Endocrinology and Metabolism, 38, 695-697.

KEOGH, H.J., JOHNSON, R.H., NANDA, R.N. and SULAIMAN, W.R. (1976). Altered growth hormone release in Huntington's chorea. Journal of Neurology, Neurosurgery and Psychiatry, 39, 244-248.

KEUL, J. and DOLL, E. (1968). The influence of exercise and hypoxia on the substrate uptake of human heart and human skeletal muscles. In: Biochemistry of Exercise, 41-46. J.R. Poortmans (Ed.). University Park Press, Baltimore.

KLAWANS, H.L. (1970). A pharmacologic analysis of Huntington's chorea. European Neurology, 4, 148-163.

KLEIN, J.P. (1966). Diphenylhydantoin intoxication associated with hyperglycaemia. Journal of Pediatrics, 69, 463-465.

KLINKERFUSS, G., BLEISCH, V., DIOSO, M.M. and PERKOFF, S.T. (1967). A spectrum of myopathy associated with alcoholism. II. Light and electron microscopic observations. Annals of Internal Medicine, 67, 493-510.

KLINTWORTH, G.K. (1973). Huntington's chorea - morphological contributions of a century. In: Advances in Neurology, 1, 353-368. A. Barbeau, T.N. Chase and G.W. Paulson (Eds.). Raven Press, New York.

KOERKER, D.J., RUCH, W., CHIDECKEL, E., PALMER, J., GOODNER, C.J., ENSINCK, J. and GALE, C.C. (1974). Somatostatin: hypothalamic inhibitor of the endocrine pancreas. Science, 184, 482-484.

KOLAKOWSKA, T., WILES, D.H., McNEILLY, H.S. and GELDER, M. (1975). Correlation between plasma levels of prolactin and chlorpromazine. Psychological Medicine, 5, 214-216.

KREUTZ, F.H. (1962). Enzymic glycerin determination. Klinische Wochenschrift, 40, 362-363.

KRIEGER, D.T. (1962). Effect of diphenylhydantoin on pituitary-adrenal interrelations. Journal of Clinical Endocrinology and Metabolism, 22, 490-493.

LAL, S., de la VEGA, C.E., SOURKES, T.L. and FRIESEN, H.G. (1973). Effect of apomorphine on growth hormone, prolactin, luteinizing hormone and follicle stimulating hormone in human serum. Journal of Clinical Endocrinology and Metabolism, 37, 719-724.

LAL, S., TOLIS, G., MARTIN, J.B., BROWN, G.M. and GUYDA, H. (1975). Effect of clonidine on growth hormone, prolactin, luteinizing hormone, follicle-stimulating hormone, and thyroid stimulating hormone in the serum of normal men. Journal of Clinical Endocrinology and Metabolism, 41, 827-832.

LANCET, (1975) Drug levels in epilepsy, ii, 264-267.

LEE, D.K., MARKHAM, C.H. and CLARK, W.G. (1968). Serotonin (5-HT) metabolism in Huntington's chorea. Life Sciences, 7, 707-712.

LEVIN, R.S., GRODSKY, G.M., HAGURA, R. and SMITH, D. (1972). Comparison of the inhibitory effects of diphenylhydantoin and diazoxide upon insulin secretion from isolated perfused pancreas. Diabetes, 21, 856-862.

LI, C.H. (1972) Hormones of the adenohypophysis. Proceedings of the American Philosophy Society, 116, 365-382.

LIEBER, C.S. (1973). Liver adaptation and injury in alcoholism. New England Journal of Medicine, 288, 356-362.

LIEBER, C.S., JONES, D. and DeCARLI, L.M. (1965). Effects of prolonged ethanol intake: production of fatty liver despite adequate diets. Journal of Clinical Investigation, 44, 1009-1021.

LOOSEN, P.T. and PRANGE, A.J. (1977). Alcohol and anterior-pituitary secretion. Lancet iii, 985.

LUCKE, C. and GLICK, S.M. (1971). Experimental modification of the sleep-induced peak growth hormone secretion. Journal of Clinical Endocrinology and Metabolism, 32, 729-736.

LUFKIN, E.G., GREENE, H.L. and MEEK, J.R. (1971). Adrenergic control of hormone secretion. Journal of Clinical and Laboratory Medicine, 78, 820.

LUFT, R. and CERASI, E. (1964). Effect of human growth hormone on insulin production in panhypopituitarism. Lancet, ii, 124-126.

LUYCKX, A.S. and LEFEBVRE, P.J. (1973). Exercise-induced glucagon secretion. Postgraduate Medical Journal, 49, 620-623.

MAANY, I., FRAZER, A. and MENDELS, J. (1975). Apomorphine: effect on growth hormone. Journal of Clinical Endocrinology and Metabolism, 40, 162-163.

MACINDOE, J.H. and TURKINGTON, P.W. (1973). Stimulation of human prolactin secretion by intravenous infusion of L-tryptophan. Journal of Clinical Investigation, 52, 1972-1978.

McLELLAN, D.L., CHALMERS, R.J. and JOHNSON, R.H. (1974). A double-blind trial of tetrabenazine, thiopropazate and placebo in patients with chorea. Lancet, i, 104-107.

MAJ, J., BARAN, L., GRABOWSKA, M. and SOWINSKA, H. (1973). Effect of clonidine on the 5-hydroxytryptamine and 5-hydroxyindoleacetic acid brain levels. Biochemical Pharmacology, 22, 2679-2683.

MALARKEY, W.B. (1976). Recently discovered hypothalamic/pituitary hormones. Clinical Chemistry, 22, 5-15.

MALHERBE, C., BURRILL, K.C., LEVIN, S.R., KARAM, J.H. and FORSHAM, R.H. (1972). Effect of diphenylhydantoin on insulin secretion in man. New England Journal of Medicine, 286, 339-342.

MANN, H.B. and WHITNEY, D.R. (1947). On a test of whether one of two random variables is stochastically larger than the other. Annals of Mathematical Statistics, 18, 52-54.

MARTIN, J.B. (1973). Neural regulation of growth hormone secretion. New England Journal of Medicine, 288, 1384-1393.

MARTIN, J.B., LAL, S., TOLIS, G. and FRIESEN, H.G. (1974). Inhibition by apomorphine of prolactin secretion in patients with elevated serum prolactin. Journal of Clinical Endocrinology and Metabolism, 39, 180-182.

MASHITER, K., ADAMS, E., BEARD, M. and HOLLEY, A. (1977). Bromocriptine inhibits prolactin and growth hormone release by pituitary tumours in culture. Lancet, ii, 197-198.

MASSARA, F. and CAMANNI, F. (1971). Plasma human growth hormone levels following the administration of various adrenergic drugs. Presented at the Second International Symposium on Growth Hormone, Milan, Italy.

MATTINGLY, D. (1962). A simple fluorimetric method for the estimation of free 11-hydroxycorticoid in human plasma. Journal of Clinical Pathology, 15, 374-379.

MELTZER, H.Y., VICTOR, M.D. and FANG, S. (1976). The effect of neuroleptics on serum prolactin in schizophrenic patients. Archives of General Psychiatry, 33, 279-286.

MERRY, J. and MARKS, V. (1969). Plasma-hydrocortizone response to ethanol in chronic alcoholics. Lancet, i, 921-923.

MERRY, J. and MARKS, V. (1972). The effect of alcohol, barbiturate and diazepam on hypothalamic/pituitary/adrenal function in chronic alcoholics. Lancet ii, 990-992.

MIMS, R.B.C., SCOTT, C.L., MODEBE, O.M. and BETHUME, J. (1973). Prevention of L-dopa induced growth hormone stimulation by hyperglycaemia. Journal of Clinical Endocrinology and Metabolism, 37, 660-663.

MORGAN, C.R. (1966). Human growth hormone immunoassay: two antibody method using ^{125}I tracer. Proceedings of the Society for Experimental Biology and Medicine, 121, 62-81.

MORGAN, C.R. and LAZAROW, A. (1963). Immunoassay of insulin; two antibody system. Diabetes, 12, 115-126.

MULLER, E.E. (1973). Nervous control of growth hormone secretion. Neuroendocrinology, 11, 338-369.

NAKAI, Y., IMURA, H., SAKURI, H., KURAHACHI, H. and YOSHIMI (1973). Effect of cyproheptadine on human growth hormone secretion. Journal of Clinical Endocrinology and Metabolism, 38, 446-449.

NICOLL, C.S. and BERN, H.A. (1972). On the actions of prolactin among vertebrates: is there a common denominator. In Lactogenic Hormones, p299. G.E.W. Wolstenholme and J. Knight (Eds.). Churchill-Livingstone, Edinburgh and London.

NICOLL, C.S. and NICHOLS, C.W. (1971). Evolutionary biology of prolactins and somatotrophins. I. Electrophoretic comparison of tetrapod prolactins. General and Comparative Endocrinology, 17, 300-310.

NOBLE, E.P. and TEWARI, S. (1977). Metabolic aspects of alcoholism in the brain. In: Metabolic Aspects of Alcoholism, 149-185. C.S. Lieber (Ed.). M.T.P. Press Ltd.

NOEL, G.L., SUH, H.K. and FRANTZ, A.G. (1971). Stimulation of prolactin release by stress in humans. Clinical Research, 19, 718.

NOEL, G.L., SUH, H.K., STONE, G. and FRANTZ, A.G. (1972). Human prolactin and growth hormone release during surgery and other conditions of stress. Journal of Clinical Endocrinology and Metabolism, 35, 840-851.

PARKES, J.D., MARSDEN, C.D., DONALDSON, I. GALEA-DELONA, A., WALTERS, J., KENNEDY, G. and ASSELMAN, P. (1976). Bromocriptine treatment in Parkinson's disease. Journal of Neurology, Neurosurgery, and Psychiatry, 39, 184-193.

PERRY, T.L., HANSEN, S. and KLOSTER, M. (1973). Huntington's chorea: deficiency of γ-aminobutyric acid in brain. New England Journal of Medicine, 288, 337-342.

PHILLIPSON, O. T. and BIRD, E.D. (1976). Plasma growth hormone concentrations in Huntington's chorea. Clinical Science, 50, 551-554.

PHILLIPSON, O.T. and BIRD, E.D. (1977). Plasma glucose, non-esterified fatty acids and amino acids in Huntington's chorea. Clinical Science, 52, 311-318.

PODOLSKY, S. and LEOPOLD, N.A. (1974). Growth hormone abnormalities in Huntington's chorea. Effect of L-dopa administration. Journal of Clinical Endocrinology and Metabolism, 39, 36-39.

PODOLSKY, S., LEOPOLD, N.A. and SAX, D.S. (1972). Increased frequency of diabetes mellitus in patients with Huntington's chorea. Lancet, i, 1356-1359.

POLISHUK, W.Z. and KULCSAR, S. (1956). Effects of chlorpromazine on pituitary function. Journal of Clinical Endocrinology and Metabolism, 16, 292-293.

RABEN, H.S. and HOLLENBERG, C.H. (1959). Effect of growth hormone on plasma fatty acids. Journal of Clinical Investigation, 38, 484-488.

RABINOWITZ, D., KLASSEN, G. and ZIERLER, K. (1965). Effect of human growth hormone in muscle and adipose tissue metabolism in the forearm of man. Journal of Clinical Investigation, 44, 51-61.

RENNIE, M.J. and JOHNSON, R.H. (1974). Alteration of metabolic and hormonal responses to exercise by physical training. European Journal of Applied Physiology, 33, 215-226.

RITCHIE, J.H. (1966). In: The Pharmacological Basis of Therapeutics, p148. L.S. Goodman and A. Gilman (Eds.). New York.

ROBERTSON, R.P. and PORTE, D. (1973). Adrenergic modulation of basal insulin secretion in man. Diabetes 22, 1-8.

ROBINSON, S. and HARMON, P.M. (1941). The lactic acid mechanism and certain properties of the blood in relation to training. American Journal of Physiology, 132, 759-769.

ROTH, J., GLICK, S.H., YALOW, R.S. and BERSON, S.A. (1963). Hypoglycaemia a potent stimulus to secretion of growth hormone. Science, 140, 987-988.

SACHDEV, Y., GOMEZ-PAN, A., TUNBRIDGE, W.M.G., DUNS, A. WEIGHTMAN, D.R. and HALL, R. (1975). Bromocriptine therapy in acromegaly. Lancet, ii, 1164-1168.

SALDANHA, V.F., HAVARD, C.W.H., BIRD, R. and GARDNER, R. (1972). The effect of chlorpromazine on pituitary function. Journal of Clinical Endocrinology, 1, 173-180.

SALTIN, B. and KARLSSON, J. (1971). Muscle ATP_1CP and lactate during exercise after physical conditioning. In: Muscle Metabolism During Exercise, B. Pernow and B. Saltin (Eds.). Plenum, New York.

SAMAAN, N.A., LEAVENS, M.E. and JESSE, R.H. (1974). Serum growth hormone and prolactin response to thyrotrophin releasing hormone in patients with acromegaly before and after surgery. Journal of Clinical Endocrinology and Metabolism, 38, 957-963.

SANBAR, S.S., CONWAY, F.J., ZWEIFLER, A.J. and SMET, G. (1967). Diabetogenic effect of Dilantin (diphenylhydantoin). Diabetes, 16, 533.

SCHALCH, D.S. (1967). The influence of physical stress and exercise on growth hormone and insulin secretion in man. Journal of Laboratory and Clinical Medicine, 69, 256-269.

SCHIMMELHISCH, W.H., MUELLER, P.S. and SCHEPS, J. (1971). The positive correlation between insulin resistance and duration of hospitalization in untreated schizophrenics. British Journal of Psychiatry, 118, 429-436.

SHAFIR, E. and KERPEL, S. (1969). Fatty acid esterification and release as related to the carbohydrate metabolism of adipose tissue; effect of epinephrine, cortisol and adrenalectomy. Archives of Biochemistry and Biophysics, 105, 237-244.

SHERMAN, L., KIM, S., BENJAMIN, F. and KOLODNY, H.D. (1971). Effect of chlorpromazine on serum growth hormone concentration in man. The New England Journal of Medicine, 284, 72-74.

SHOULSON, I., KARTZINEL, R. and CHASE, T.N. (1976). Huntington's disease: treatment with diprophylacetic acid and gamma-aminobutyric acid. Neurology, 26, 61-63.

SILER, T.M., VANDENBERG, G., YEN, S.S.C., BRAZEAU, P., VALE, W. and GUILLEMIN, R. (1973). Inhibition of growth hormone release in humans by somatostatin. Journal of Clinical Endocrinology and Metabolism, 37, 632-634.

SINGER, C.J. (1952). Vesalius on the human brain. Oxford University Press, London.

SMITH, P.E. (1930) Hypophysectomy and replacement therapy in the rat. American Journal of Anatomy, 45, 205-273.

SMYTHE, G.A. and LAZARUS, L. (1974). Suppression of human growth hormone secretion by melatonin and cyproheptadine. Journal of Clinical Investigation, 54, 116-121.

SOURKES, T.L. (1975). Neural and neuroendocrine functions of dopamine. Psychoneuroendocrinology, 1, 69-78.

SPIRO, S.G., JUNIPER, E., BOWMAN, P. and EDWARDS, R.H.T. (1974). An increasing work rate test for assessing the physiological strain of submaximal exercise. Clinical Science, 46, 191-206.

STAHL, W.L. and SWANSON, P.D. (1974). Biochemical abnormalities in Huntington's chorea brains. Neurology (Minneap.), 24, 813-819.

STRAUCH, G., MODIGLIANI, E. and BRICAIRE, H. (1968). Growth hormone response to arginine in normal and hyperthyroid females under propanolol. Journal of Clinical Endocrinology, 29, 606-608.

SUOMINEN, H., FORSBERG, S., HEIKKINEN, E. and OSTERBACK, L. (1974). Enzyme activities and glycogen concentration in skeletal muscle in alcoholism. Acta Medica Scandinavica, 196, 199-202.

SUTTON, J., YOUNG, J., LAZARUS, L., HICKIE, J. and MAKSUYTIS, J. (1968). Hormonal changes during exercise. Lancet, 2, 1304.

TAMBURRANO, G., TAMBURRANO, S., GAMBARDELLA, S. and ANDREANI, D. (1976). Effects of alcohol on growth hormone secretion in acromegaly. Journal of Clinical Endocrinology and Metabolism, 42, 193-195.

THORNER, M.O., CHAIT, A., AITKEN, M., BENKER, M., BLOOM, S.R., MORTIMER, C.H., SANDERS, P., STUART-MASON, A. and BESSER, G.M. (1975). Bromocriptine treatment in acromegaly. British Medical Journal, 1, 299-303.

TOLIS, G., PINTER, E.J., and FRIESEN, H.G. (1975). The acute effect of 2-bromo-α-ergocriptine (CB-154) on anterior pituitary hormones and free fatty acids in man. International Journal of Clinical Pharmacology, 12, 281-283.

TORO, G., KOLODNY, R.C., JACOBS, L.S., MASTERS, W.H. and DAUGHADAY, W.H. (1973). Failure of alcohol to alter pituitary and target organ hormone levels. Clinical Research, 21, 505.

TURKINGTON, R.W., UNDERWOOD, L.E. and VAN WYK, J.J. (1971). Elevated serum prolactin levels after pituitary stalk section in man. New England Journal of Medicine, 285, 707-710.

TYSON, J.E., KHOJANDI, M., HUTH, J. and ANDREASSEN, B. (1975). The influence of prolactin secretion on human lactation. Journal of Clinical Endocrinology and Metabolism, 40, 764-773.

UNGER, R.H. and LEFEBVRE, P.J. (1972). Glucagon physiology. In: Glucagon, 213-244. R.H. Unger and P.J. Lefebvre (Ed.) Pergamon Press, Oxford.

VAISBERG, M. and SAUNDERS, J.C. (1963). Treatment of dyskinesias including Huntington's chorea with thiopropazate and R1625. Diseases of the Nervous System, 24, 499-500.

WEIR, G.C., TURNER, R.C. and MARTIN, D.B. (1973). Glucagon radioimmunoassay using antiserum 30K: interference by plasma. Hormone and Metabolic Research, 5, 241-244.

WERNER, W.H., REY, H.G. and WIELINGER, H. (1970). Uber die eigenshaften eines neuen chromogens fur die blutzuckerbestimmung nach der GOD/POD methode. Zeitschrift Analytische Chemie, 252, 224-228.

WIDE, L. (1969). Radioimmunoassays employing immuno-sorbents. Acta Endocrinologica, 63, supplement 142, 207-218.

WILES, D.H., KOLAKOWSKA, T., McNEILLY, A.S., MANDELBROTE, B.M. and GELDER, M.G. (1976). Clinical significance of plasma chlorpromazine levels. 1. Plasma levels of the drug, some of its metabolites and prolactin during acute treatment. Psychological Medicine, 6, 407-415.

WILLIAMSON, D.H., MELLANBY, J. and KREBS, H.A. (1962). Enzymatic determination of D(-)-β-hyroxybutyrate and acetoacetic acid in blood. Biochemical Journal, 82, 90-96.

WINKLER, B., STEELE, R. and ALTSZULER, N. (1969). Effects of growth hormone administration on free fatty acid and glycerol turnover in the normal dog. Endocrinology, 85, 25-30.

WOODBURY, D.M. (1952). Effects of chronic administration of anticonvulsant drugs, alone and in combination with deoxycorticosterone, on electroshock seizure threshold and tissue electrolytes. Journal of Pharmacology and Experimental Therapeutics, 105, 46-57.

WRIGHT, J.W., FRY, D.E., MERRY, J. and MARKS, V. (1976). Abnormal hypothalamic-pituitary-gondal function in chronic alcoholics. British Journal of Addiction, 71, 211-215.

YALLOW, R.S. and BERSON, S.A. (1960). Immunoassay of endogenous plasma insulin in man. Journal of Clinical Investigation, 39, 1157-1175.

YEN, S.S.C., SILER, T.M. and DE VANE, G.W. (1974). Effect of somatostatin in patients with acromegaly. New England Journal of Medicine, 290, 935-938.

ZACUR, H.A., FOSTER, G.V. and TYSON, J.E. (1976). Multi-factorial regulation of prolactin secretion. Lancet, i, 410-412.

PUBLICATIONS AND COMMUNICATIONS

The following publications and communications have been made of the results presented in this thesis.

CHALMERS, R.J., JOHNSON, R.H. and SULAIMAN, W.R. (1977). The metabolic response to exercise in chronic alcoholics. Quarterly Journal of Experimental Physiology, 35, 261-269.

CHALMERS, R.J., BENNIE, E.H., JOHNSON, R.H. and KINNELL, H.G. (1977). Growth hormone response to insulin induced hypoglycaemia in alcoholics. Clinical Science, 52, 26P.

CHALMERS, R.J., BENNIE, E.H., JOHNSON, R.H. and KINNELL, H.G. (1977). The growth hormone response to insulin induced hypoglycaemia in alcoholics. Psychological Medicine, 7, 607-611.

CHALMERS, R.J., JOHNSON, R.H. and NANDA, R.N. (1977). Growth hormone and prolactin release in Huntington's chorea. Lancet ii, 824.

CHALMERS, R.J., JOHNSON, R.H. and NANDA, R.N. (1977). Growth hormone and prolactin response to bromocriptine in patients with Huntington's chorea. Journal of Neurology, Neurosurgery and Psychiatry (in press).

CHALMERS, R.J., BENNIE, E.H. and JOHNSON, R.H. (1977) Effect of fluphenazine on pituitary function in man. Clinical Endocrinology (in press).

CHALMERS, R.J. and BENNIE, E.H. (1977). The effect of fluphenazine on basal prolactin concentrations Psychological Medicine (in press).

CHALMERS, R.J., BENNIE, E.H., JOHNSON, R.H. and MASTERTON, G. (1977). Growth hormone, prolactin and cortisol response to insulin hypoglycaemia in alcoholics. British Medical Journal (in press).