GROWTH HORMONE RELEASING PEPTIDES: PHYSIOLOGICAL STUDIES IN MAN

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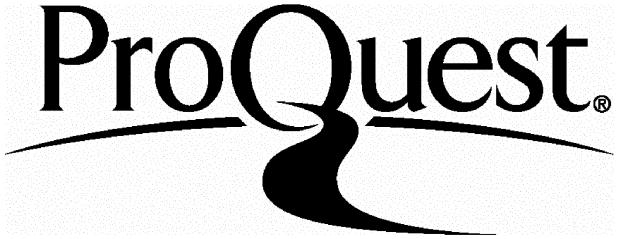

ABSTRACT

This work describes physiological studies of the growth hormone releasing peptide (GHRP) hexarelin. Four groups of studies were conducted on young, healthy, adult male volunteers, as follows:

1. Studies which demonstrated that hexarelin is a potent growth hormone (GH) secretagogue, capable of inducing GH release after two successive administrations. GHRP acts synergistically with growth hormone releasing hormone (GHRH) to induce massive GH release. However, this synergistic action is lost on repeated administration.

2. Studies which showed that GH itself attenuates the GH response to GHRP.

3. Studies which showed that the GH-releasing activity of GHRP, alone or in combination with GHRH, is attenuated but not abolished by increasing somatostatin (SS) tone. The large GH release induced by GHRP plus GHRH despite the presence of high SS tone suggests that combined therapy may be utilised to produce GH release in conditions where SS tone is unknown.

4. Studies which established GH dose-response curves for hexarelin. This group of studies also showed that hexarelin is non-specific for GH release, inducing prolactin (PRL) and cortisol release in a dose-related manner. These studies showed that at low doses of hexarelin it is possible to induce adequate GH release with minimum concomitant rise in PRL and cortisol. Moreover, combined GHRH plus low dose hexarelin is synergistic for GH release with no additive effect on PRL and cortisol release.

The actions of GHRPs and their interaction with the two main endogenous regulators of GH secretion, namely, GHRH and SS, strongly suggests that an endogenous GHRP ligand exists and plays an important role in the regulation of GH release. The specific GH release induced by low doses of GHRP, alone or in combination with GHRH, together with the oral activity of these peptides, holds a great promise for future therapeutic use.

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LIST OF ABBREVIATIONS

ACTH	Adrenocorticotrophic hormone
Aib	α-aminoisobutyryl
Ala	Alanine
ANOVA	Analysis of variance
AUC	Area under the curve
b.d.	Twice daily
BMI	Body mass index
cAMP	Cyclic adenosine monophosphate
CNS	Central nervous system
CV	Coefficient of variance
D	D-stereoisomer
Da	Dalton
ED50	The dose which would be expected to produce 50% of the maximal response
FFA	Free fatty acids
FSH	Follicle stimulating hormone
GH	Growth hormone
GHRH	Growth hormone releasing hormone
GHRP	Growth hormone releasing peptide
Gly	Glycine
gm	Gram
GnRH	Gonadotrophin releasing hormone
GTP	Guanylyl triphosphate
His	Histidine
h	Clock hour
hr	Hour
HV	Height velocity
IGF-1	Insulin-like growth factor-1
IGF-BP3	Insulin-like growth factor binding protein-3
IGHC	Integrated growth hormone concentration
i.n.	Intranasal
irGHRP	Immunoreactive growth hormone releasing peptide
ITT	Insulin tolerance test
IU	International units
i.v.	Intravenous
kg	Kilogram
L	Litre
L	L-stereoisomer
Leu	Leucine
LH	Luteinizing hormone
LHRH	Luteinizing hormone releasing hormone
LPH	Lipotropin
L-type	Long-lasting-type
Lys	Lysine

Met	**Methionine**
mg	**Milligram**
min	**Minute**
ml	**Millilitre**
MRI	**Magnetic resonance imaging**
mRNA	**Messenger ribonucleic acid**
mU	**Milliunit**
Nal	**Naphthyl-alanine**
NETRIA	**North East Thames Region Immunoassay**
ng	**Nanogram**
NS	**Not significant ($p > 0.05$)**
PACAP	**Pituitary adenylate cyclase-activating polypeptide**
Phe	**Phenylalanine**
Pit-1	**Pituitary-specific transcription factor-1**
p.o.	**Oral**
PRL	**Prolactin**
r	**Spearman's rank correlation coefficient**
r-hGH	**Recombinant human growth hormone**
RIA	**Radioimmunoassay**
s.c.	**Subcutaneous**
SD	**Standard deviation**
SDS	**Standard deviation score**
SEM	**Standard error of the mean**
SPSS	**A statistics software package**
SS	**Somatostatin**
SS20	**Somatostatin infusion at a rate of 20 $\mu g/m^2/hr$**
SS50	**Somatostatin infusion at a rate of 50 $\mu g/m^2/hr$**
T3	**Triiodothyronine**
T4	**Thyroxine**
t.d.s.	**Three times daily**
TRH	**Thyrotropin releasing hormone**
Trp	**Tryptophan**
TSH	**Thyroid stimulating hormone**
T-type	**Transient-type**
Tyr	**Tyrosine**
VIP	**Vasoactive inhibitory peptide**
vs	**Versus**
μg	**Microgram**
/	**Per**

ACKNOWLEDGEMENTS

First and foremost I would like to acknowledge my two supervisors, Professor Charles Brook and Dr Peter Hindmarsh. I was very fortunate to have worked in Professor Brook's Department as a Senior House Officer and to have had exposure to Paediatric Endocrinology early on in my postgraduate training. It was at that stage in my career that I became interested in Paediatric Endocrinology. I was inspired by Professor Brook to pursue this interest and it was he who gave me the opportunity to undertake clinical and, later, research training in this fascinating field of medicine. I returned to his Department and worked in various capacities (Registrar, Research Fellow and Senior Registrar). He has supported me throughout my years of General and Higher Specialist Training and has always provided invaluable clinical, academic and personal advice. Words cannot express my thanks to him.

I have been extremely fortunate to work with and be supervised by Dr Peter Hindmarsh. As well as providing first-class research advice and up-to-date scientific information, he has been a constant source of inspiration, guidance and support. He never failed to find time to discuss research issues, analyse and interpret data or solve problems. His assistance with the more complex statistical analyses was greatly appreciated. His brilliant ideas and his love and enthusiasm for generating sound scientific data maintained the rapid momentum with which the GHRP project started. My utmost thanks and gratitude to him for his efforts and hard work.

I am most grateful to Professor Mike Preece for his involvement in the final stages of this work. He kindly took on the laborious task of supervising my writing-up process. He provided constructive criticism and invaluable practical advice. His support at this very critical stage of the project was greatly appreciated.

I also wish to acknowledge Dr David Matthews for his help in performing the deconvolution analysis on my data, Ms Jane Pringle and her laboratory assistants for co-ordinating and performing the assays, the LCPEM Clinical Nurse Specialists for their assistance in collecting blood specimens, staff of the UCL Illustration Centre for producing the graphics and the

volunteers for agreeing to take part in the study.

I cannot submit this thesis without a special word of thanks to my parents for their long-standing unconditional support. Without their firm belief in good quality education and the importance of advancing knowledge, together with their selflessness and willingness to invest in me, I would not have got to where I am today. I thank them for having faith in me. I am forever indebted to them.

Finally but most importantly, I wish to thank my wife Samah who witnessed only the final stages of the experimental work but lived through the writing-up process in its entirety. Without her moral support, her relentless encouragement and her expert computer skills this work would never have been completed. I thank her for making it happen.

DECLARATION OF INVOLVEMENT

All the studies presented in this thesis were designed and executed by myself. I alone recruited all the volunteers. All sampling sessions were co-ordinated by myself. This included labelling of test-tubes, setting up of equipment and facilities, and administration of drugs. The specimens were drawn by myself, with occasional assistance from the Clinical Nurse Specialists working at the London Centre for Paediatric Endocrinology and Metabolism. I was responsible for spinning, separating and storing the samples.

Because of the large number of samples collected, my involvement in performing the various assays was limited. These were co-ordinated by Miss PJ Pringle in the Cobbold Laboratories. I did, however, perform all the blood glucose measurements.

I was assisted in the mathematical processing for the deconvolution analysis by Dr David Matthews, The Radcliffe Infirmary, Oxford. The data analysis, their presentation and interpretation are otherwise entirely my own.

AIMS OF THESIS

This thesis addresses issues relating to the potential physiological role and therapeutic application of growth hormone releasing peptides (GHRPs), a new group of growth hormone (GH) secretagogues. Four groups of studies were conducted on young, healthy, adult male volunteers. The studies were designed to answer several questions relating to the actions and properties of GHRPs, their interaction with GH, growth hormone releasing hormone (GHRH) and somatostatin (SS). The purpose of these studies was:

1. To determine the effect of single and repeated doses of a novel GHRP, hexarelin, administered alone or in combination with GHRH, on GH secretion. The effect of single and repeated doses of GHRH alone on GH secretion was also determined and compared with the effect of GHRP alone.

2. To investigate the effect of GH itself on the GH response to GHRP.

3. To investigate the effect of varying SS tone and the effect of SS withdrawal on the GH response to GHRP, alone or in combination with GHRH. For comparison, the GH response to GHRH alone was studied under the same experimental conditions.

4. To determine the GH dose-response curve for hexarelin and to investigate the effect of this GHRP on other hormones, namely, prolactin, cortisol, TSH and insulin. The effect on blood glucose was also studied. Combination studies of low dose GHRP plus GHRH were conducted.

CHAPTER 1

INTRODUCTION

Background

Human growth hormone (GH) is essential for normal linear growth and has a number of endocrine, metabolic and immunologic effects. GH is released from the anterior pituitary gland in a pulsatile manner and is under the control of a number of neurotransmitters and neuropeptides as well as various hormones and metabolic products. The main factors which control GH synthesis and secretion are the two neuropeptides growth hormone releasing hormones (GHRH) and somatostatin (SS).

GHRH was isolated in 1982 from a pancreatic tumour in a patient with acromegaly. Extensive studies have shown that it acts directly at the pituitary somatotroph via a specific receptor to induce GH synthesis and secretion via cyclic adenosine monophosphate (cAMP). SS, on the hand, inhibits GH secretion but does not affect its synthesis. The interaction of the two neuropeptides and their asynchronous periodic release from the hypothalamus are largely responsible for the pulsatile nature of GH secretion.

The development in the late 1970s of a group of synthetic potent GH secretagogues, which became known as the growth hormone releasing peptides (GHRPs), introduced another dimension into the control of GH secretion. The first GHRP that was synthesised was originally thought to be GHRH itself. As information about GHRPs accumulated it became apparent that it was not. Researchers established that the two neuropeptides differed in many respects, most importantly in the receptor sites on which they acted. The potent GH-releasing property of GHRPs and, more importantly, their activity following oral administration generated a great deal of interest in this area of endocrine research. The search for an endogenous GHRP-like ligand commenced and is still, at the time of writing, on-going.

The purpose of this thesis was to study the physiology of hexarelin, a novel growth hormone releasing peptide, by conducting studies which would address some important issues relating to the interaction of GHRPs with other factors which control GH secretion. Such studies would pave the way for the obvious next step, namely, therapeutic trials.

Section 1. The Growth Hormone Releasing Peptide Story - a Historical Review

The birth of growth hormone releasing peptides

As new brain peptides are isolated and their chemical structures sequenced they become potential candidates for hypothalamic hypophysiotropic hormones, especially if they have the general hallmarks of the native hypophysiotropic hormones, namely, peptide structure, distribution within the hypothalamus and possession of multiple biological activities, including extrapituitary effects.

In 1975, Hughes and co-workers isolated from porcine brain two enkephalin pentapeptides, Tyr-Gly-Gly-Phe-Met (Met^5-enkephalin) and Tyr-Gly-Gly-Phe-Leu (Leu -enkephalin) (Hughes *et al.*, 1975). These were shown to exhibit the biological activities of opiates. Detailed *in vitro* studies of the hypophysiotropic activities of these two peptides revealed that, at pharmacological dosages (10-100 μg/ml incubation medium), they inhibited the Luteinizing Hormone (LH) and Follicle Stimulating Hormone (FSH) response to Luteinizing Hormone Releasing Hormone (LHRH), and that the amides of these peptides had agonist LH/FSH activity at the same high concentrations (Bowers *et al.*, 1977a). An interesting aspect of Met^5-enkephalin is that its amino acid sequence is identical to residues 61-65 of the pituitary hormone β-lipotropin (β-LPH), and that the sequence Tyr-Gly-Gly-Phe-Met is identical to the N-terminus of the powerful brain opiate peptide β-endorphin (Chretien *et al.*, 1976; Li *et al.*, 1976).

In 1977, Bowers *et al* (1977b) developed an analogue of Met^5-enkephalin, Tyr-DTrp-Gly-Phe-Met-NH_2, (DTrp^2), a methionine enkephalin amide, and showed that it too had hypophysiotropic properties. Bowers and co-workers demonstrated that DTrp^2 was capable of releasing GH but not Thyroid Stimulating Hormone (TSH), LH, FSH, prolactin (PRL), or

adrenocorticotrophic hormone (ACTH) *in vitro*, albeit at relatively high concentrations (1-20 μg/ml incubation medium) (Bowers *et al.*, 1977a; Bowers *et al.*, 1977b). DTrp^2 was thus the first reported peptide that acted on the pituitary to release only GH. The "discovery" of DTrp^2 was to become a breakthrough in the development of what later became known as growth hormone releasing peptides (GHRPs).

The development of growth hormone releasing peptides

In the years that followed, Bowers, Momany and co-workers conducted studies to understand the structural requirements of Tyr-DTrp-Gly-Phe-Met-NH_2 to release GH *in vitro* (Bowers *et al.*, 1980). They did so by synthesizing analogues of the molecule with either single or multiple amino acid substitutions and determining their *in vitro* GH-releasing activity. A number of interesting findings were reported. Substitution of an aromatic D residue at the 2 position was of prime importance for GH-releasing activity; both Tyr-DTrp-Gly-Phe-Met-NH_2 and Tyr-DPhe-Gly-Phe-Met-NH_2 released GH *in vitro*. In contrast, the LTrp analogue had no GH-releasing activity, thus indicating the importance of a D amino acid residue at the 2 position. C-terminal amidation considerably enhanced the GH-releasing activity of the peptide. Neither opiates nor their antagonist, naloxone, inhibited the GH release induced by Tyr-DTrp-Gly-Phe-Met-NH_2. Of all the peptides studied at the time, the latter remained the most potent for GH release (Bowers *et al.*, 1980).

The above work was extended to include studies of the structure-activity relationship of the enkephalin analogues and their hypophysiotropic *in vitro* GH-releasing activity. Decisions for designing the chemical modifications were made on the basis of what was believed to be the low energy GH-releasing conformation of Tyr-DTrp-Gly-Phe-Met-NH_2. An approach was developed in which the information from conformational energy calculations, calculated theoretically by Momany (Bowers *et al.*, 1980; Momany *et al.*, 1981), was used to search for structural features common to the active analogues. This was followed by designing several new peptides whose structural changes, as determined from conformational analysis, enhanced the conformational properties thought to be responsible for activity. New analogues were synthesized, and then biological activities were determined *in vitro*. The biological activity

data were then used to further refine the understanding of the binding and the mechanism of action of the peptides at the cellular level. The cycle of design, synthesis, and testing for biological activity was repeated with the new series of analogues (Momany *et al.*, 1981).

The above approach to peptide design differed in two aspects from the classical method of modifying the activity of endogenous peptide hormones by means of treating the linear sequence of amino acids as simple substitution sites. First, no endogenous peptide hormone had (or has) been isolated. Thus an attempt was being made to drive a relatively non-potent GH-releasing peptide into the physiological range without prior knowledge that this was possible. Second, the insertion of conformational energy calculations as structural data into the design cycle both before synthesis as well as after the activity-testing step was not used in the classical analogue approach.

The work of Momany *et al* (1981) established that a very specific configuration of selected aromatic rings was required of all peptides found to release GH specifically. The work culminated in the development of a new and more potent analogue, Tyr-DTrp-Ala-Trp-DPhe-NH_2, capable of releasing GH *in vitro* at 10-30 ng/ml medium, which was approximately 10^3 times more active than the two starting enkephalin-based analogues. However, Tyr-DTrp-Ala-Trp-DPhe-NH_2 had no *in vivo* activity (Momany *et al.*, 1984).

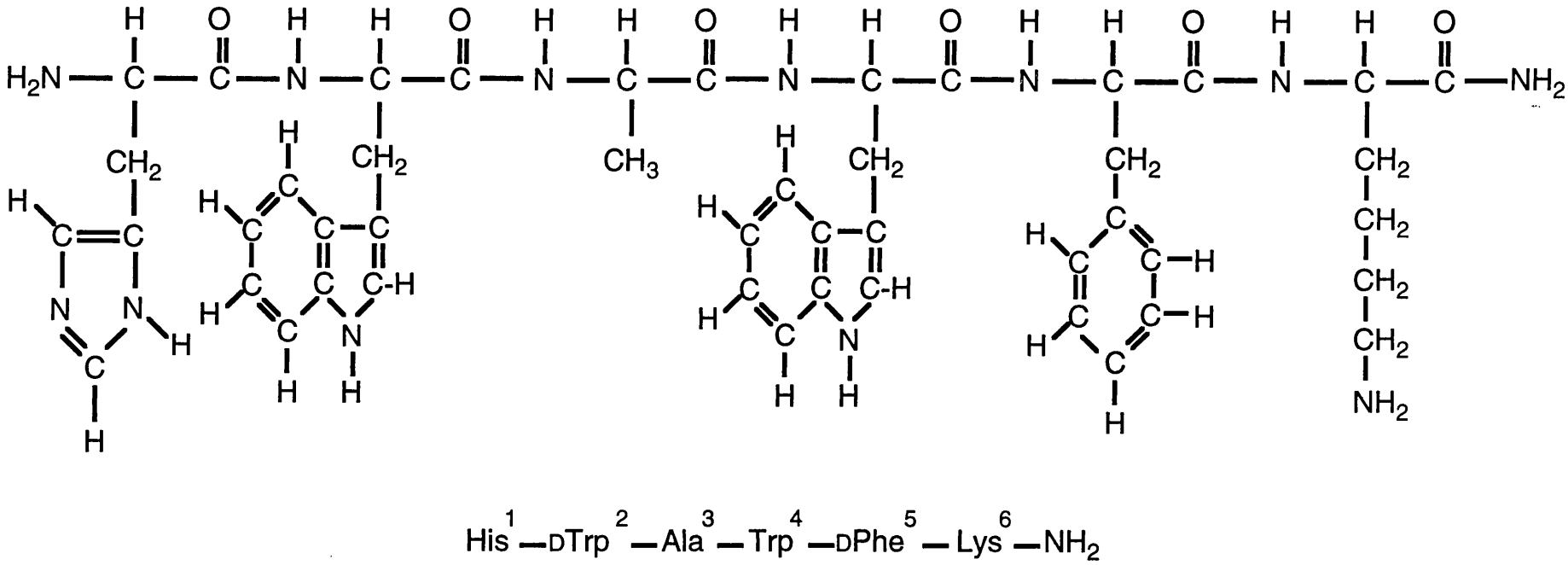
In subsequent studies, Momany *et al* (1984) found that substituting histidine for tyrosine in Tyr-DTrp-Ala-Trp-DPhe-NH_2 was favourable for *in vivo* activity. In addition to acquiring *in vivo* activity, the resulting molecule His-DTrp-Ala-Trp-DPhe-NH_2 was found to have *in vitro* activity at approximately 3 ng/ml medium. Progressively greater amounts of GH were released with 10 and 30 ng/ml (Momany *et al.*, 1984). Extending the molecule by adding lysine to the C-terminus gave a more active peptide, His-DTrp-Ala-Trp-DPhe-Lys-NH_2. This peptide was active *in vitro* at 1 ng/ml. *In vivo* activity, tested in immature female rats, for both molecules was dose-dependent, with His-DTrp-Ala-Trp-DPhe-Lys-NH_2 active at approximately 1 μg/rat subcutaneously (s.c.). Modifications of the first histidine ring did not alter the GH-releasing activity, whereas the two tryptophan rings appeared to be necessary. The phenylalanine ring was also sensitive to modification.

The term Growth Hormone Releasing Peptide was coined in 1984 (Momany *et al.*, 1984; Bowers *et al.*, 1984). In the same year, Bowers and co-workers reported the specific GH-releasing activity of His-DTrp-Ala-Trp-DPhe-Lys-NH_2 *in vitro* and *in vivo* in multiple animal species (rhesus monkeys, lambs, calves, rats and chicks) and by various routes of administration (intravenous, subcutaneous and intraperitoneal) (Bowers *et al.*, 1984). Since His-DTrp-Ala-Trp-DPhe-Lys-NH_2 was active in the rhesus monkey, this strongly supported the possibility that the peptide may be active in humans. Coupled with its potency, specificity and absence of untoward effects on various biochemical and haematological parameters in animal studies, it became an obvious choice for further development. His-DTrp-Ala-Trp-DPhe-Lys-NH_2 later became known as GHRP-6 (Figure 1). The suffix 6 was given because it consisted of six amino acids (Bowers, personal communication).

GHRP-6 was extensively studied over the following decade. Further *in vitro* and *in vivo* studies of GHRP-6 were conducted to elucidate its site and mechanism of action and its interaction with various factors which control GH secretion. Particular emphasis was made on its interaction with GHRH and SS, as well as the effects of its acute and chronic administration.

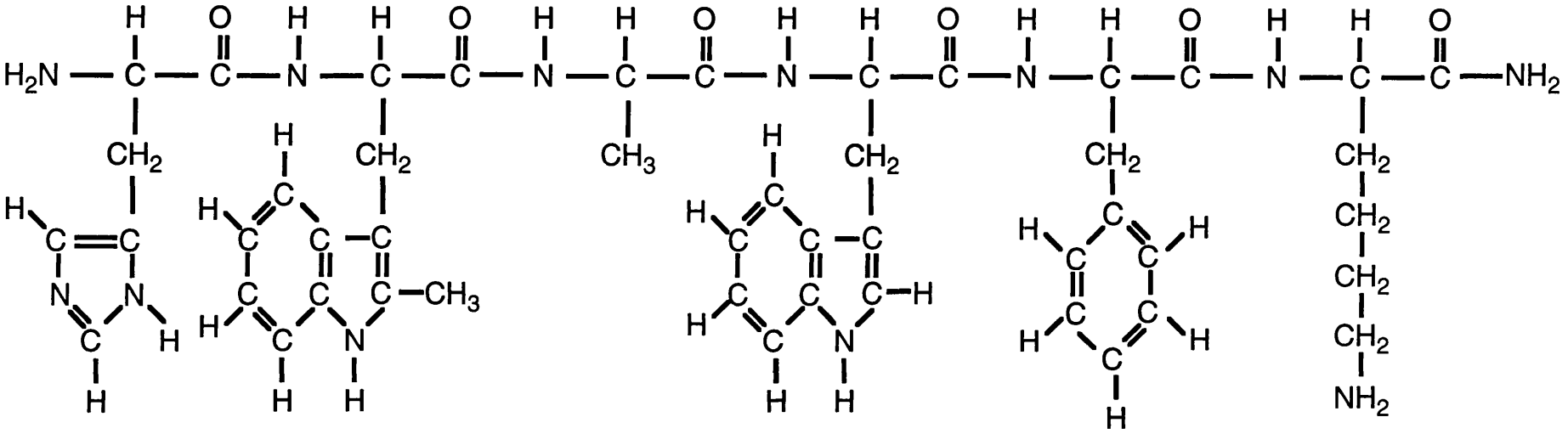
In the late 1980s, the more potent GHRP-1 heptapeptide (Ala-His-DβNal-Ala-Trp-DPhe-Lys-NH_2) (DβNal = beta naphthyl-D-alanine) and GHRP-2 hexapeptide (DAla-DβNal-Ala-Trp-DPhe-Lys-NH_2) were developed (Bowers *et al.*, 1991a; Bowers, 1993a). Another GHRP, hexarelin (His-D2-methylTrp-Ala-Trp-DPhe-Lys-NH_2) (Figure 2), which differs from GHRP-6 by a single methyl group substitution at the second position of the indole ring of the DTrp residue, was developed in 1992 (Deghenghi *et al.*, 1992; Deghenghi *et al.*, 1994).

The clinical era of the GHRPs began in 1988. Since then, clinical studies have been greatly extended using different types of GHRPs by various routes of administration. The acute and chronic effects of the various GHRPs on GH release have been, and continue to be, investigated as a function of age and pathophysiological secretion of GH. Studies of the diagnostic and therapeutic uses of GHRPs have commenced recently.



GHRP-6

Figure 1. Chemical structure of GHRP-6.



His[1]—D2-methyl Trp[2]—Ala[3]—Trp[4]—DPhe[5]—Lys[6]—NH_2

Hexarelin

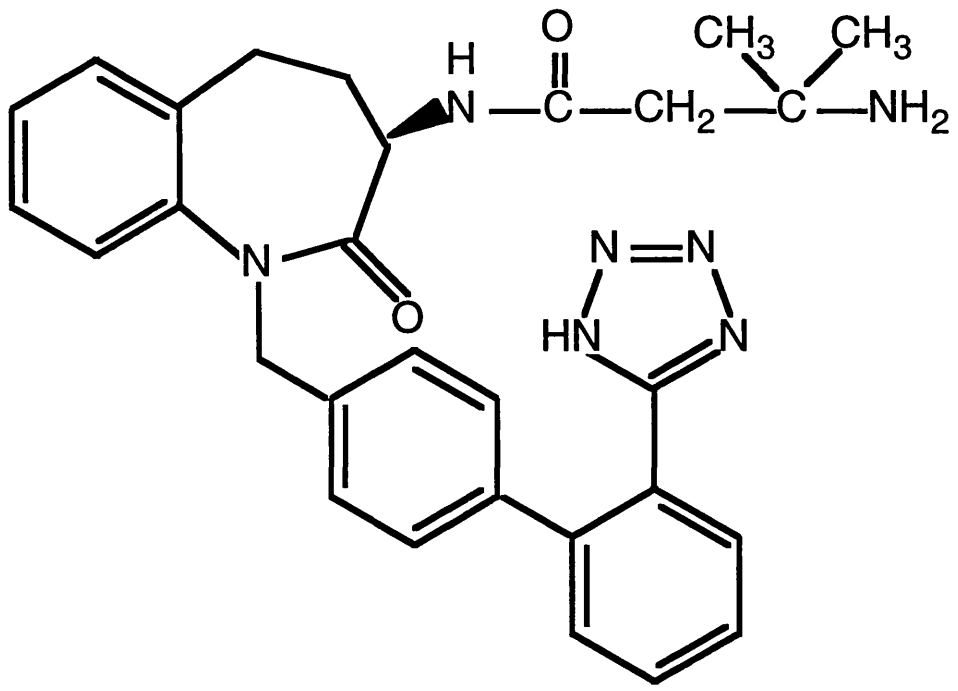
Figure 2. Chemical structure of hexarelin.

Non-peptidyl and low molecular weight peptide growth hormone secretagogues

Noteworthy has been the recent development of a series of peptidomimetic or non-peptidyl GH secretagogues (Smith *et al.*, 1993; Cheng *et al.*, 1993; Bowers *et al.*, 1994). These were modeled on GHRP-6, using it as a template to screen various structural classes for GH-releasing activity.

Smith *et al* (1993) sought small nonpeptide mimetics because they provide the structural diversity necessary to allow a molecule to be optimised for specificity, oral bioavailability and pharmacokinetic properties. The work of Bowers and Momany and co-workers (Bowers *et al.*, 1984, Momany *et al.*, 1984) led Smith and co-workers (Smith *et al.*, 1993) to conclude that the aromatic amino acid residues and the NH_2-terminal amine were important for bioactivity. On the basis of this hypothesis, a diverse group of structures which contained these features were selected and their capacity specifically to induce GH release was tested. The first compound to be identified (L-158,077) consisted of a benzolactam ring with an amino acid side chain, an aromatic ring and a biphenyl scaffold. L-158,077 had modest, but specific, GH releasing activity *in vitro*.

By substituting the carboxylic acid function of the first phenyl ring of L-158,077 with a tetrazole, a dramatic increase in potency was observed. The resultant compound, L-692,429 (Figure 3), {[3(R)-amino-3-methyl-N-(2,3,4,5-tetrahydro-2-oxo-1)-(2'-(1H-tetrazol-5-yl)-(1,1'-biphenyl)4-yl][methyl-1H-1-benzazepin-3yl]butanamide,mono(hydrochloride), dihydrate}, was found to have the same biological activities as GHRP-6 (Smith *et al.*, 1993; Gertz, *et al.*, 1993). This accomplishment is of considerable scientific importance because a small organic chemical compound has been developed which has the agonist, rather than the antagonist, activity of a small peptide. Further modification of these small organic compounds resulted in the development of a closely related nonpeptide, L-692,585, that is 10-20 times more potent than L-692,429 (Jacks *et al.*, 1994). The most recently developed potent and orally active non-peptidyl GH secretagogue, L-163,191 (MK-0677), a spiroindoline, has been



L-692,429

Figure 3. Structure of the non-peptidyl GH secretagogue L-692,429.

selected for clinical studies (Patchett *et al.*, 1995, Copinshi *et al.*, 1996, Chapman *et al.*, 1996).

Development of novel classes of peptides that stimulate GH secretion by re-examination of the structure/function of GHRP-6 continues (Elias *et al.*, 1995, Deghenghi *et al.* 1995). Elias *et al* (1995) reported the development of four novel classes of GH-releasing peptides; a pentapeptide G-7039 [isonipecotinyl-DβNal-DβNal-Phe-Lys-NH_2; molecular weight 797 daltons (Da)], a tetrapeptide G-7134 [isonipecotinyl-DβNal-DβNal-N-(2-phenylethyl)glycine; molecular weight 683 Da], a pseudotripeptide G-7502 (isonipecotinyl-DβNal-D-tryptophanol; molecular weight 498 Da) and a rigid cyclic heptapeptide G-7203 [cyclo-D-Lys-DβNal-Ala-Trp-D-Phe-(ethyl)Lys-NH_2; molecular weight 986 Da]. The latter is a cyclic analogue of GHRP-2. In comparison, the molecular weight of GHRP-6 is 872 Da.

As these novel GHRPs differ dramatically in size and structure, it was necessary to determine whether GHRP potency and specificity were retained (Elias *et al.*, 1995). These compounds were found to have potent, dose-dependent GH-releasing activity. They synergised with GHRH but not GHRP-6, and demonstrated homologous desensitization after continuous exposure while maintaining sensitivity to GHRH. Somatostatin inhibited all of these compounds. All four classes elicited a small increase in PRL but no change in LH, FSH, ACTH or TSH. Additionally, G-7039 elevated intracellular free calcium, as occurs with GHRP-6. These attributes suggest that the action of all four compounds is mediated via a specific GHRP-like mechanism.

The design of small, low molecular weight GHRPs whilst maintaining GH-releasing potency has tempted workers because of the potential improved bioavailability via several routes (e.g. oral and pulmonary). Tetralin, a tetrapeptide (γ-aminobutyryl-D2-methylTrp-DβNal-Phe-Lys-NH_2) developed by Deghenghi *et al* (1995), has been found to have potent *in vitro* and *in vivo* activity. Down-sizing of hexarelin to the shorter tripeptide Aib-D2-methylTrp-D2-methylTrp-NH_2 (Aib = α-aminoisobutyryl) has resulted in greater GH-stimulating activity (Deghenghi *et al.*, 1996). The low molecular weight GHRP G-7502 reported by Elias *et al* (1995) has been shown to retain the *in vitro* and *in vivo* activity of GHRP-6 (McDowell *et al.*, 1995).

The potency and small size of these molecules make them excellent candidates for drug development.

Section 2. The Pharmacology of Growth Hormone Releasing Peptides

Structure

GHRPs are synthetic peptides consisting of a small number (≤ 7) of amino acids. The first potent GHRP with *in vivo* activity was GHRP-6. To date, it is the most extensively studied of all GHRPs. The amino acid sequences of the four GHRPs (GHRP-6, GHRP-1, GHRP-2 and hexarelin) so far given to man, are as follows:

GHRP-6: His-DTrp-Ala-Trp-DPhe-Lys-NH_2

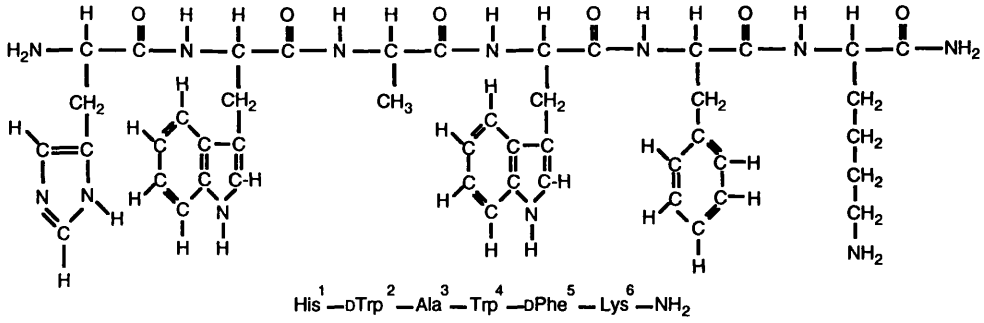
GHRP-1: Ala-His-DβNal-Ala-Trp-DPhe-Lys-NH_2

GHRP-2: DAla-DβNal-Ala-Trp-DPhe-Lys-NH_2

Hexarelin: His-D2-methylTrp-Ala-Trp-DPhe-Lys-NH_2

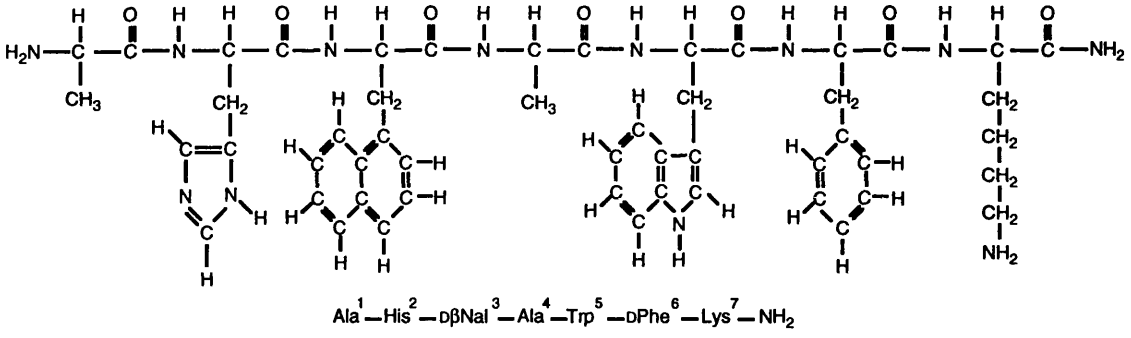
(The chemical structures of the four peptides are shown in Figure 4).

The important chemical characteristics which enhance the bioactivity of these peptides are: small size, D-amino acid stereoisomers, aromatic side chain rings with a spacer (alanine) between them and the presence of a lysine residue at the C-terminus (Momany *et al.*, 1984; Bowers, 1993a). GHRP-1 and GHRP-2 have the addition of an alanine at the N-terminus and the unnatural beta naphthyl-D-alanine with the more hydrophobic bicyclic aromatic side chain ring instead of the D-tryptophan residue in GHRP-6. Hexarelin is an analogue of GHRP-6, in which the second tryptophan residue has been replaced by its more hydrophobic and



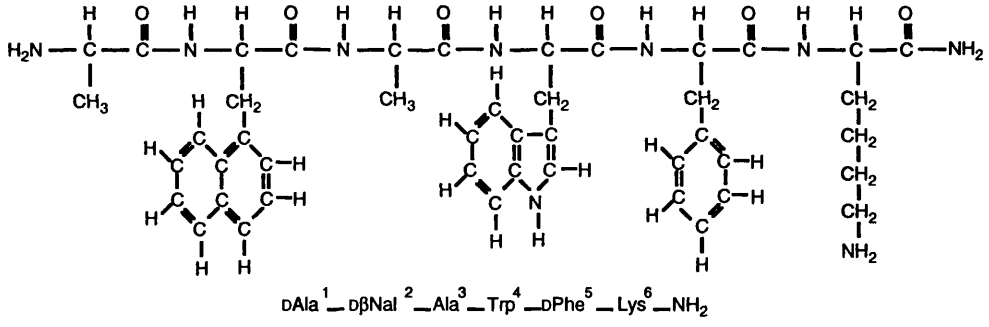
His[1]—DTrp[2]—Ala[3]—Trp[4]—DPhe[5]—Lys[6]—NH_2

GHRP-6



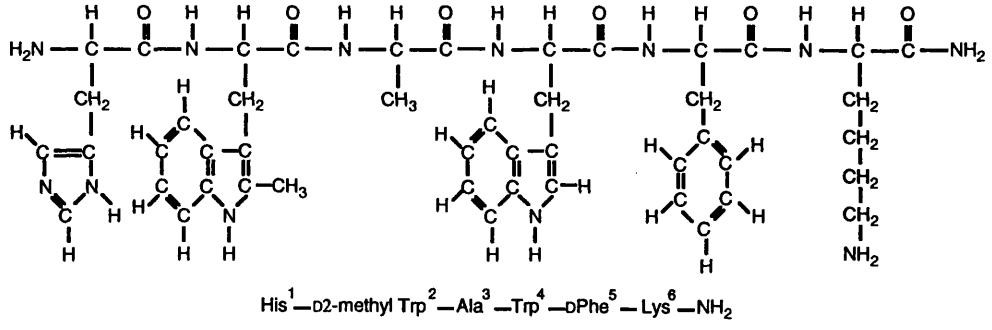
Ala[1]—His[2]—DβNal[3]—Ala[4]—Trp[5]—DPhe[6]—Lys[7]—NH_2

GHRP-1



DAla[1]—DβNal[2]—Ala[3]—Trp[4]—DPhe[5]—Lys[6]—NH_2

GHRP-2



His[1]—D2-methyl Trp[2]—Ala[3]—Trp[4]—DPhe[5]—Lys[6]—NH_2

Hexarelin

Figure 4. Chemical structure of GHRP-6, GHRP-1, GHRP-2 and hexarelin.

chemically more stable 2-methylated derivative (Deghenghi *et al.*, 1992). It has a molecular weight of 888 Da (Imbimbo, personal communication). Substitution of the second tryptophan residue with the L isomer of 2-methyl tryptophan resulted in lesser potency (Deghenghi *et al.*, 1992), confirming the earlier observations of Bowers and Momany that the D stereoisomer was essential for enhanced bioactivity (Bowers *et al.*, 1980).

There have been no studies comparing in a standardised manner the relative potency of all four GHRPs. Subcutaneous GHRP-2 is more potent than subcutaneous GHRP-1. GHRP-1 is in turn more potent than GHRP-6 (Bowers, 1993a). Animal studies have shown that subcutaneous hexarelin is more potent than subcutaneous GHRP-6 (Deghenghi *et al.*, 1994).

It is worth noting that GHRPs have no sequence homology with GHRH which consists, in its natural forms, of 44, 40 or 37 amino acids (Dieguez *et al.*, 1988). GHRH is structurally related to the glucagon-secretin class of gut hormones, which includes vasoactive inhibitory peptide (VIP) and gastric inhibitory peptide, and is present in the hypothalamus and the gastrointestinal tract. The *in vitro* and *in vivo* biological activity of GHRH resides in the N terminal 29 amino acid residues (Rivier *et al.*, 1982) and the presence of tyrosine or histidine in position one is essential for receptor binding (Ling *et al.*, 1984). Attempts to synthesise shorter analogues have resulted in loss of potency (Wehrenberg and Ling, 1983).

Synthesis

GHRP-6 was originally synthesized by the solid state method (Stewart and Young, 1968). Benzhydryl amino resin was used as the solid support and the crude peptide was cleaved from it by the standard hydrogen fluoride method (Stewart and Young, 1968). The peptide was purified by partition chromatography. The purity of the peptide was determined by high performance liquid chromatography, amino acid analysis, and silica gel thin layer chromatography (Bowers *et al.*, 1984). Similarly, hexarelin was also synthesized by conventional solid phase synthesis (Deghenghi *et al.*, 1994).

Activity and bioavailability

GHRPs are active following intravenous (i.v.), subcutaneous (s.c.), intranasal (i.n.) and oral (p.o.) administration (Bowers *et al.*, 1984; Ilson *et al.*, 1989; Walker *et al.*, 1990; Nelson *et al.*, 1991; Hartman *et al.*, 1992; Bowers, 1993a; Ghigo *et al.*, 1994a, Laron *et al.*, 1994). The bioavailability of GHRPs by the different routes varies enormously. Data on bioavailability are somewhat limited and are based largely on comparison with the GH response in man to a "standard" dose of the peptide (1.0 μg/kg) given intravenously. Those published are shown in Table 1. Animal studies have shown similar data, with the bioavailability of GHRP-6 in rats, dogs and monkeys following enteral (intragastric) administration being 0.7%. Monkeys were the species most sensitive to parenteral (intravenous) and enteral (intragastric) GHRP-6 administration (Walker *et al.*, 1990).

Table 1. Bioavailability of GHRPs following various routes of administration.

Route	GHRP-6	GHRP-1	GHRP-2	hexarelin
s.c.				68 - 86%[1]
i.n.	5%[2]		8%[3]	4 - 6%[1]
p.o.	0.3%[4,5]	0.15%[6]		0.2 - 0.3%[1]

[1] Ghigo *et al.*, 1994a

[2] Hayashi *et al.*, 1991

[3] Pihoker *et al.*, 1995a

[4] Hartman *et al.*, 1992

[5] Bowers *et al.*, 1992

[6] Bowers, 1993a

Dosage

The fact that GHRPs released GH in a dose-dependent manner was established early on in the development of the peptides, both *in vitro* and in *in vivo* animal studies (Momany *et al.*, 1981; Bowers *et al.*, 1984; McCormick *et al.*, 1985; Bowers *et al.*, 1991b; Deghenghi *et al.*, 1994). Further studies showed this to be the case in humans too (Ilson *et al.*, 1989; Bowers *et al.*, 1990; Hayashi, *et al.*, 1991; Hartman, *et al.*, 1992, Bowers *et al.*, 1992). Although the GH responses to various individual doses of GHRPs administered by various routes have been studied, there remains a paucity of published data on dose-response curves of the GH-releasing activity of the various GHRPs.

The i.v. route has been the most extensively used method of administration in animal and human studies. However, the maximal effective i.v. doses of various GHRPs, with the exception of hexarelin, have not been fully evaluated. In human studies, i.v. doses of 1.0 μg of GHRP per kilogram (kg) of body weight are thought to induce near-maximal GH responses. Indeed, the majority of i.v. GHRP studies in humans, with the exception of hexarelin, have been conducted using doses of 1.0 μg/kg (see Table 2). When i.v. doses of 2.0 μg of GHRP per kg of body weight administered to normal adults were compared with 1.0 μg/kg doses, the increase in peak serum GH concentration was slight and statistically not significant (Ghigo *et al.*, 1994a). A dose-response study of hexarelin by Imbimbo *et al* (1994) showed this increase to be approximately 6%. Although i.v. doses of hexarelin up to 2.0 μg/kg were well tolerated in man, phase 1 studies in adult volunteers reported that transient flushing, the most frequent adverse effect of the drug, was dose-related and happened more commonly at the 2.0 μg/kg dose (R Deghenghi, personal communication).

Table 2. Peak serum GH concentrations following i.v. administration of GHRP-6, GHRP-1, GHRP-2 and hexarelin to normal young adult humans.

GHRP	Dosage	no. of subjects	Peak serum GH concentration (mean ± SEM)	Reference
GHRP-6	1.0 μg/kg	17	63.0 ± 5.4 μg/L	Ilson *et al.*, 1989
GHRP-6	1.0 μg/kg	10	26.0 ± 5.6 μg/L	Hartman *et al.*, 1992
GHRP-6	1.0 μg/kg	6	68.7 ± 15.5 μg/L	Bowers *et al.*, 1990
GHRP-6	1.0 μg/kg	6	54.9 ± 4.2 μg/L	Hayashi *et al.*, 1991
GHRP-6	1.0 μg/kg	5	29 ± 4.5 μg/L	Bowers *et al.*, 1992
GHRP-6	1.0 μg/kg	25	37.2 ± 6.6 mU/L	Peñalva *et al.*, 1993a
GHRP-6	100 μg	5	38.7 ± 7.6 mU/L	Leal-Cerro *et al.*, 1994
GHRP-6	90 μg	11	28.3 ± 6.0 μg/L	Popovic *et al.*, 1995
GHRP-6	1.0 μg/kg	9	23.2 ± 3.9 μg/L	Ramos-Dias *et al.*, 1996
GHRP-1	1.0 μg/kg	10	54.8 ± 5.3 μg/L	Bowers, 1993a
GHRP-2	1.0 μg/kg	8	109 ± 80.6 mU/L *	Tiulpakov *et al.*, 1995a
hexarelin	1.0 μg/kg	12	52.3 ± 5.0 ng/mL	Imbimbo *et al.*, 1994
hexarelin	2.0 μg/kg	12	55.0 ± 4.5 ng/mL	Imbimbo *et al.*, 1994
hexarelin	2.0 μg/kg	7 6	72.0 ± 7.1 μg/L 57.9 ± 8.6 μg/L	Arvat *et al.*, 1994 Arvat *et al.*, 1994
hexarelin	2.0 μg/kg	6 6	69.1 ± 7.4 μg/L 62.6 ± 8.0 μg/L	Arvat *et al.*, 1995 Arvat *et al.*, 1995
hexarelin	2.0 μg/kg	5	48.8 ± 6.3 μg/L	Cappa *et al.*, 1995
hexarelin	2.0 μg/kg	6	62.6 ± 8.0 μg/L	Maccario *et al.*, 1995
hexarelin	2.0 μg/kg	18	90.8 (50.6-181) mU/L#	Ciccarelli *et al.*, 1996
hexarelin	2.0 μg/kg	6	67.3 ± 7.4 μg/L	Arvat *et al.*, 1996

* mean ± SD

median (range)

The s.c., i.n. and oral dosages have been based largely on comparisons with the responses to the i.v. dosages. The administration of a 3.0 μg/kg s.c. dose of GHRP-1 in normal men and women resulted in a peak GH response similar to that following an i.v. dose of 1.0 μg/kg of the same peptide (Bowers, 1993a). The peak GH response to a 1.0 μg/kg s.c. dose of GHRP-2 was similar in magnitude to that following a 3.0 μg/kg s.c. dose of GHRP-1, confirming the greater potency of the former (Bowers, 1993a). Ghigo *et al* (1994a) found a dose-related effect after s.c. administration of hexarelin to adult men and women, though the responses to only two different doses were reported (1.5 and 3.0 μg/kg). The GH response to 3.0 μg/kg s.c. was similar to that obtained following 1.0 μg/kg i.v. A trend towards a lower responsiveness in women was found which may be related to differences in subcutaneous fat distribution in the two sexes. There are no published data on the s.c. dosage of GHRP-6.

Marked GH release occurred in normal men when they were given 30 μg/kg i.n. GHRP-6 (Hayashi *et al.*, 1991). Ghigo *et al* (1994a) administered 20 μg/kg hexarelin i.n. to normal men and women. The peak GH response was submaximal (approximately 80% of the response to 1.0 μg/kg i.v.). Laron *et al* (1994), however, obtained similar peak GH responses when they administered 20 μg/kg hexarelin i.n. and 1.0 μg/kg i.v. to a group of children (aged 5.5-15.5 years) with familial short stature. Pihoker *et al* (1995a) observed a dose-related response to i.n. GHRP-2, over a dose-range of 5-20 μg/kg, in children with short stature.

Despite the very low bioavailability of oral GHRP, many workers have shown that near-maximal GH responses can be obtained by this route of administration, the feature that holds the greatest promise for future therapeutic uses. Bowers (1993a) showed that oral doses of GHRP-1 of 300 or 600 μg/kg were almost as effective as the intravenous dose of 1.0 μg/kg in terms of GH response in normal adults (Bowers, 1993a). A similar phenomenon was observed with GHRP-6 (Hartman *et al.*, 1992; Bowers *et al.*, 1992). Studies of oral hexarelin in normal adults by Ghigo *et al* (1994a) showed that the GH response to 20 mg (~ 270 μg/kg) was similar to that reported with 300 μg/kg GHRP-6. In the same study, a higher dose of oral hexarelin (40 mg, approximately 720 μg/kg) was administered to women and resulted in a significantly greater GH response. The authors estimated the ED_{50} (the dose which would be expected to produce 50% of the maximal response) of hexarelin by the oral route to be

approximately 500 μg/kg.

Characteristics of the growth hormone response to growth hormone releasing peptides

The characteristics of the GH response to the various GHRPs are fairly similar, with some variation according to the route of administration. Following i.v. administration of a GHRP, there is a rapid (within 10 minutes) rise in serum GH, which peaks within 30-40 minutes and returns to basal levels by 180-240 minutes. The onset of the GH rise is slower following s.c. and i.n. administration (approximately 15 minutes), and is slowest following oral administration (15-30 minutes). Similarly, the time to reach peak GH concentration is longer following s.c. and i.n. administration (40-60 minutes), and is longest following oral administration (60-75 minutes). The characteristics of the GH response to GHRP-6, GHRP-1, GHRP-2 and hexarelin are shown in Tables 3-6.

Table 3. Characteristics of the GH response to GHRP-6 following various routes of administration.

Route of administration	i.v.	i.n.	p.o.
Dose (μg/kg)	1.0	30	300
Time to start rising (minutes)	3#	<15*	30#
Time to peak concentration (minutes)	30-45#	45*	60-75#
Time to return to basal levels (minutes)	180#	180*	150-180#
Peak GH response (μg/L) (mean ± SEM)	29 ± 4.5#	39.6 ± 15.3*	30 ± 4.0#

Bowers *et al.*, 1992

*Hayashi *et al.*, 1991

Table 4. Characteristics of the GH response to GHRP-1 (Bowers, 1993a) following various routes of administration.

Route of administration	i.v.	s.c.	p.o.
Dose (μg/kg)	1.0	3.0	600
Time to start rising (minutes)	<5	15	15-20
Time to peak concentration (minutes)	30-40	40-60	60
Time to return to basal levels (minutes)	180-240	180-240	180-240
Peak GH response (μg/L) (mean ± SEM)	54.8 ± 5.3	55.0 ± 6.3	31.8 ± 5.3

Table 5. Characteristics of the GH response to GHRP-2 following various routes of administration.

Route of administration	i.v.	s.c.
Dose (μg/kg)	1.0	1.0
Time to start rising (minutes)	<10*	15#
Time to peak concentration (minutes)	30*	40-60#
Time to return to basal levels (minutes)	120*	240-300#
Peak GH response	109.7± 80.6 mU/L (mean ± SD)*	52 ± 5 μg/L (mean ± SEM)#

Bowers, 1993a

*Tiulpakov *et al.*, 1995a

Table 6. Characteristics of the GH response to hexarelin (Ghigo *et al.*, 1994a) following various routes of administration.

Route of administration	i.v.	s.c.	i.n.	p.o.
Dose	1.0 μg/kg 2.0 μg/kg	1.5 μg/kg 3.0 μg/kg	20 μg/kg	20 mg 40 mg
Time to start rising (minutes)	<15	<15	<15	<15
Time to peak (minutes) (mean ± SEM)	28 ± 3, 24 ± 2	43 ± 4, 46 ± 3	35 ± 4	64 ± 9, 51 ± 5
Time to return to basal levels (minutes)	180	180	180	180
Peak GH response (μg/L) (mean ± SEM)	51.5 ± 6.5, 61.7 ± 8.1	48.9 ± 6.2, 57.9 ± 5.4	40.5 ± 7.2	31.8 ± 5.7, 54.7 ± 6.6

Measurement of serum growth hormone releasing peptide

Specific radioimmunoassays (RIA) for measurement of serum immunoreactive GHRP (irGHRP-6 or irGHRP-1) levels have been developed by Tulane Endocrine Laboratory (Tulane, New Orleans, USA), and have been used to investigate the pharmacokinetics of GHRPs in some studies (Bowers *et al.*, 1992; Bowers, 1993a). The sensitivity of the assay was determined to be 0.5 μg/L; intra- and inter-assay coefficients of variation were 9.1% and 13%, respectively.

The first report of serum irGHRP levels in humans was by Bowers *et al.* (1992). In their study of normal adults they found that, following three different dosages (0.1, 0.3 and 1.0 μg/kg) of i.v. bolus GHRP-6, the mean peak serum irGHRP-6 levels were proportionally

related to the dose (~8, 15, 40 μg/L). The rise in serum irGHRP-6 level was immediate, with a rapid attainment of peak levels. Serum irGHRP-6 levels fell at a gradually decreasing exponential rate over the first 100 minutes. The calculated serum half-life of GHRP-6 was 20 minutes, with an initial volume of distribution of 2.5L, indicating that it is mainly limited to the blood volume. Following i.v. administration the mean serum irGHRP-6 level remained constant and slightly elevated between 100 and 240 minutes. Following an oral GHRP-6 dose of 300 μg/kg, given to a similar group of men, an elevation of serum irGHRP-6 was measurable 15 minutes after ingestion. The peak rise in serum irGHRP-6 level (~20 μg/L) occurred at 60 minutes. The initial serum half-life was 20 minutes, with an initial volume of distribution of 2.5L. Serum irGHRP-6 remained elevated for the 5 hour period of observation. The kinetics for i.v. and p.o. GHRP-6 were similar in a group of children with short stature studied by the same authors.

In a similar study by Bowers (1993a), the half-life for serum irGHRP-1 following i.v. GHRP-1 (1.0 μg/kg) was calculated to be 20 minutes with a volume of distribution of 2.5L. Following an oral dose of 600 μg/kg of the same peptide, serum irGHRP-1 started to rise after 10 minutes, peaked at 45 minutes and gradually returned to baseline by 5 hours. The mean peak serum irGHRP-1 following i.v. (1.0 μg/kg), s.c. (3.0 μg/kg) and p.o. (600 μg/kg) GHRP-1 were 9.7, 16.0 and 5.1 μg/L, respectively. Interestingly, once the serum irGHRP-1 concentration reached 10 μg/L, the release of GH did not increase further. This finding mirrored that seen *in vitro*, where 10 μg/L of GHRP-1 produced maximal GH release when added to pituitary dispersed rat culture cells, and confirmed the high potency of the peptide (Bowers, 1993a).

A highly sensitive and specific RIA has been developed for hexarelin (Roumi *et al.*, 1995). The sensitivity of the assay was determined to be 1.34 fmol/assay (2.68 pmol/L, equivalent to 2.38 nanograms/L); intra- and inter-assay coefficients of variation were less than 3% and 4%, respectively. Cross-reactivity of the antiserum with nine hexarelin analogues was less than 1%. There was no cross-reaction with endogenous hexarelin metabolites. This RIA has been used to study the pharmacokinetics of hexarelin in dogs following an i.v. dose of 1.0 μg/kg and three s.c. doses of 1, 10 and 100 μg/kg. Intravenous bolus pharmacokinetics of

hexarelin displayed a high terminal half-life of 120 minutes, a fractional plasma clearance of 4.28 ml/min/kg and a volume of distribution of 387.7 ml/kg. Following s.c. administration of hexarelin of increasing doses of hexarelin, both clearance (3.93-5.17 ml/min/kg) and volume of distribution (316-544 ml/kg) parameters remained constant over the dose range (Roumi *et al.*, 1995). There are no reports of serum hexarelin measurements in humans to date.

Chronic administration of growth hormone releasing peptide

Most studies of GHRPs in human have looked at the effect of acute bolus administration of the peptides. A few researchers have investigated the effect of chronic GHRP administration, either in the form of continuous i.v. infusion or repeat bolus administration.

a) Infusion studies

DeBell *et al* (1991) investigated the effect of a continuous 6-hour i.v. GHRP-6 infusion in 6 healthy young (20-34 years) subjects. Three doses of GHRP-6 were infused (0.1, 0.3, 1.0 μg/kg/hr) and an i.v. bolus of GHRP-6 (1.0 μg/kg) was administered 5.5 hours after the start of the infusion. For each dose of the 6-hour GHRP infusion a single burst of GH release occurred, though sporadic secretory episodes of lesser magnitude were observed with the highest infusion dose. The magnitude of this initial peak increased with increasing GHRP infusion dosage. Linear regression analysis demonstrated a significant relationship between the GHRP infusion dose and GH secretion rates ($r = 0.81$; $p < 0.001$). With each dose, peak GH concentration occurred 50-120 minutes (mean: 84 minutes) after initiation of the GHRP infusion. Bolus i.v. administration of GHRP-6 5.5 hours after the start of the infusion resulted in a second GH peak, which was lowest following the high GHRP-6 infusion rate (1.0 μg/kg/hr). Peak GH concentrations occurred 10-50 minutes (mean: 35 minutes) after the administration of the i.v. bolus. The mean secretion rate in response to the i.v. bolus was inversely related to the infused dose of GHRP-6 ($r = -0.58$; $p = 0.003$), indicating a partial response attenuation. Interestingly, the total amount of GH secreted (constant infusion plus

bolus periods) was not different among study days, and there were no dose-related changes in secretion.

Huhn *et al* (1993) studied the effect of a 24-hour i.v. GHRP-6 infusion (1.0 μg/kg/hr) in 8 healthy adults (24-32 years). Saline infusions were administered to the same subjects for comparison. Thirty minutes before the end of the infusion an i.v. bolus of GHRP-6 or GHRH was administered. Each subject completed four study sessions: (i) saline infusion, GHRP bolus, (ii) saline infusion, GHRH bolus, (iii) GHRP infusion, GHRP bolus, and (iv) GHRP infusion, GHRH bolus. During GHRP infusion, mean integrated GH concentrations (IGHC; min.μg/L) and GH secretion rates were increased 8 fold. Single bursts of GH release occurred shortly after the start of the GHRP infusions and peaked between 60-130 minutes (mean: 82 minutes). Initial bursts were followed by GH pulses of lower magnitude. GH pulse number, duration, and height; incremental pulse amplitude; and interpeak valley concentration were significantly greater, and interpulse intervals were significantly shorter during the GHRP-6 infusion compared to those during saline treatment. A most interesting finding in this study was the significant correlations between the two GHRP-6 infusions in the same individual for several attributes of pulsatile GH release, including IGHC, number of pulses, pulse height, incremental pulse amplitude, interpeak valley concentration and individual pulse area. Enhancement of GH secretion by GHRP-6 infusion was highly reproducible ($r = 0.77$; $p = 0.004$). The bolus administration of GHRP-6 or GHRH 30 minutes from the end of the infusion resulted in an enhanced GH secretion in all treatment groups. However, the GH responses to GHRP-6 were attenuated and those to GHRH were enhanced after continuous GHRP-6 infusion as compared to their respective responses after saline infusion. This study demonstrated that pulsatile GH release was consistently enhanced by continuous exposure to GHRP and that the response to bolus GHRP after 24 hour exposure to GHRP was partially attenuated.

Jaffe *et al* (1993) carried out a study to determine whether humans would remain responsive to prolonged exposure to GHRP. In a similar design to Huhn's study (see above), 34 hour i.v. infusions of GHRP-6 (1.0 μg/kg/hr) or saline were administered to 9 healthy adults (20-42 years). As with the above studies prolonged GHRP infusion led to a significant augmentation

of spontaneous GH secretion as well as to an increase in GH responsiveness to GHRH. Similarly, the GH response to i.v. GHRP bolus during GHRP infusion was significantly smaller than the response to GHRP bolus given during saline infusion, a finding that is consistent with the desensitization seen after a 6-hour (DeBell *et al.*, 1991) and a 24-hour GHRP infusion (Huhn *et al.*, 1993).

b) Repeat bolus administration

Hayashi *et al* (1991) administered 7 consecutive i.n. doses of GHRP-6 to 5 healthy adults. Doses of 15 μg/kg every 8 hours were used. There was no attenuation of the plasma GH response to GHRP-6. Although there was no statistical difference in the GH response after the first and the seventh GHRP-6 administration, the GH response seemed to be rather enhanced after repeated administration.

Ghigo *et al* (1996a) investigated the short term effect of repeated i.n. administration of hexarelin in normal elderly subjects (67-80 years, n = 7). The acute GH responses to i.n. hexarelin (approximately 18 μg/kg, 8 hourly) were maintained after 8 days of treatment. Similarly, the administration of oral hexarelin (approximately 300 μg/kg, 8 hourly) to another 7 elderly subjects (63-80 years) did not result in desensitization after 15 days of treatment.

The effect of repeated administration of i.n. hexarelin in 7 constitutionally short prepubertal children was investigated by Frankel *et al* (1995). Three one-week trials with increasing doses of hexarelin (20 μg/kg b.d., 20-20-40 μg/kg and 40 μg/kg t.d.s.), with a one week washout between each dosage regimen, were carried out. Serum GH levels were not documented but serum insulin-like growth factor-1 (IGF-1) increased significantly after each one week trial.

In a short-term (8 month follow-up) therapeutic trial of hexarelin in 8 short normal prepubertal children, i.n. administration (60 μg/kg) of the secretagogue three times daily resulted in a significant increase in the mean linear growth velocity. Growth velocity increased from 5.4 ± 0.8 (mean ± SD) cm/year before treatment to 8.3 ± 1.7 cm/year after eight months of treatment ($p < 0.0001$). Serum GH levels were not documented but serum

IGF-1 increased significantly after treatment (Laron *et al.*, 1995a).

Interaction of growth hormone releasing peptides with growth hormone releasing hormone and somatostatin

It was a natural step in the investigation of GHRPs to study their interaction with the two key factors which control GH secretion. Consequently, there is a wealth of literature on the interaction of GHRPs with GHRH and, to a lesser extent, on their interaction with SS. Because most work in this area was primarily performed to elucidate the mechanism of action of GHRPs, it is covered in Section 3 of this Chapter. However, it is prudent to mention at this stage two important interactions: the first is the attenuation, but not abolition, of the GH response to GHRP by SS (Arvat *et al.*, 1995). The second is the synergistic *in vivo* GH releasing action of GHRP and GHRH. Synergism of GHRP and GHRH is referred to when the amount of GH released following their combined administration is more than additive, i.e. is significantly greater than the sum of the amounts of GH released following their isolated administration. Bowers *et al* (1990) demonstrated this phenomenon in 7 normal young men when they showed that the peak GH response to GHRP-6 (0.1 μg/kg) plus GHRH (1.0 μg/kg) was 80.0 ± 14.6 μg/L, to GHRP-6 (0.1 μg/kg) alone was 7.6 ± 2.5 μg/L and to GHRH (1.0 μg/kg) alone was 36.0 ± 3.9 μg/L. This finding has been reproduced by many workers and for all four GHRPs given to man so far (Bowers *et al.*, 1992; Bowers 1993a; Tiulpakov *et al.*, 1995a; Pihoker *et al.*, 1995a; Micic *et al.*, 1995).

Effect of sex and age on the action of growth hormone releasing peptides

a) Sex effect

Published data indicate that, for similar age groups, there is no difference in the GH response to GHRPs between males and females (Peñalva *et al.*, 1993a; Cordido *et al.*, 1993; Laron

et al., 1993; Loche *et al.*, 1995a; Ramos-Dias, 1996), except for a slightly lower responsiveness to hexarelin in adult females following s.c. administration (Ghigo *et al.*, 1994a) and greater responsiveness in pubertal females following i.v. administration (Bellone *et al.*, 1995b). The former may be related to differences in s.c. fat distribution in the two sexes, while the latter may be due to differences in serum oestradiol levels. Physiological changes in plasma oestrogen levels in women during the menstrual cycle did not cause any differences in the GH response to GHRP-6 (Peñalva *et al.*, 1993a), though the GH responses in pubertal girls correlated with serum oestradiol concentration ($r = 0.57$; $p < 0.03$) (Bellone *et al.*, 1995b).

b) Age effect

Once it was established that GHRPs were potent GH secretagogues in healthy adult humans, it was natural to proceed with investigating their activity in different age groups.

(i) Studies of growth hormone releasing peptides in normal prepubertal children

Because of the ethical problems of investigating and venesecting healthy children most of these studies were carried out on so-called short normal children, though some workers were able to study children of normal stature (Peñalva *et al.*, 1993a). All four GHRPs have been administered intravenously to prepubertal children. In addition, GHRP-6 has been administered orally and hexarelin intranasally.

All studies showed that normal prepubertal children were capable of responding to GHRP stimulation. Although some studies included only a small number of children (Tiulpakov *et al.*, 1995b; 5 children) others were much larger (Bellone *et al.*, 1995b; 34 children). Most children reported in the various studies were in mid-childhood (7-11 years). The youngest reported was 5.8 years (Laron *et al.*, 1994). One study included children who had constitutional delay of growth and puberty (Tiulpakov *et al.*, 1995b). Table 7 summarises the data published so far on studies of GHRPs in normal prepubertal children.

Table 7. Studies of GHRPs in prepubertal children.

GHRP	Route of administration	Dosage	no. of subjects	Age range (years)	Peak serum GH concentration (mean ± SEM)	Reference
GHRP-6	i.v.	1.0 μg/kg	6 M, 6 F	7-11	M: 57 ± 11.2 , F: 44.2 ± 5.4 mU/L	Peñalva *et al.*, 1993a*
GHRP-6	i.v.	1.0 μg/kg	12	7-11	25.3 ± 3.4 μg/L	Pombo *et al.*, 1995a
GHRP-6	p.o.	300 μg/kg	13	6.2-10.5	18.8 ± 3.0 μg/L	Bellone *et al.*, 1995a
GHRP-1	i.v.	1.0 μg/kg	6	9.4 ± 0.45**	20.2 ± 5.0 μg/L	Laron *et al.*, 1993
GHRP-2	i.v.	1.0 μg/kg	5	6.9-13.6	41.8 (range: 8.7-104.0) μg/L	Tiulpakov *et al.*, 1995b
hexarelin	i.v.	1.0 μg/kg	6	5.8-11.3	range: 30-150 mU/L	Laron *et al.*, 1994
	i.n.	20 μg/kg	6	5.8-11.3	range: 30-140 mU/L	Laron *et al.*, 1994
hexarelin	i.v.	2.0 μg/kg	34	11.2 ± 0.4**	range: 8.5-130.3 μg/L	Bellone *et al.*, 1995b
hexarelin	i.v.	2.0 μg/kg	24	5.9-13	47 ± 4.0 μg/L	Loche *et al.*, 1995a

* Children in this study were of normal stature.

** (mean ± SEM)

M: male; F: female

(ii) Studies of growth hormone releasing peptides in pubertal children

The effect of puberty on the GH response to hexarelin was studied by Bellone *et al* (1995b). Fifty two prepubertal and 44 pubertal subjects (puberty stage II-IV) were studied. Although 47 subjects had short stature, this was familial as indicated by their otherwise normal auxological, biochemical and endocrine data. Subjects were given i.v. doses of hexarelin (2.0 μg/kg) which elicited a greater increase in GH levels ($p < 0.001$) in pubertal than prepubertal children. The maximum serum GH concentration in the two groups ranged from 24.3 to 158.3 μg/kg and from 8.5 to 130.3 μg/kg, respectively. Interestingly, with advancing puberty, the GH-releasing effect of hexarelin increased more in girls than in boys ($p < 0.05$). The GH responses to hexarelin correlated with oestradiol concentration ($r = 0.57$; $p < 0.03$) in girls, but not with testosterone concentration in boys.

Loche *et al* (1995a) studied 21 children in early puberty (Tanner stage 2-3, age 10-14 years) and 24 prepubertal children and found no difference in the peak GH response to i.v. hexarelin (2.0 μg/kg) between the two groups. In the same study, five subjects with constitutional delay of growth and puberty were treated with testosterone for one week. The peak GH response to hexarelin was significantly greater after priming (57.4 ± 8.7 μg/L *vs* 89.2 ± 11.3μg/L). In contrast to the findings by Bellone *et al* (1995b), there were no differences in the GH responses in male and female pubertal subjects in this study.

Laron *et al* (1993) studied the GH response to i.v. GHRP-1 in 9 adolescents (mean age 15.4 ± 0.45 years). Peak serum GH concentration was 35.5 ± 8.4 μg/L, greater than that in prepubertal subjects (see Table 7).

(iii) Studies of growth hormone releasing peptides in the elderly

Both spontaneous and stimulated GH secretion in middle and late adulthood decline with age. The reduction in pulsatile GH release, the reduced responses to GHRH and the decrease in the plasma concentrations of IGF-1 with advancing age may contribute to the changes in body mass composition that occurs with ageing (Finkelstein *et al.*, 1972; Shibasaki *et al.*,

1984; Corpas *et al.*, 1993). These changes can be reversed to some extent by exogenous GH administration (Rudman *et al.*, 1990). These findings prompted many researches to investigate the actions of GHRPs in normal ageing in an attempt to gain further insight into the disrupted GH secretion in late adulthood and to investigate the potential use of GHRPs in reversing these changes.

Micic *et al* (1995) studied a group of 9 healthy young adults (22 ± 1.1 years; mean ± SEM) and 9 older subjects (59.5 ± 1.7 years). The mean age difference was approximately 37 years. Each subject was given an i.v. bolus of GHRH (100 μg/kg), GHRP-6 (90 μg/kg) and GHRH plus GHRP-6 in the same above doses. While the peak serum GH response to GHRH was significantly higher in the young subjects, the peak GH responses to GHRP-6 and GHRP-6 plus GHRH were similar in the two groups. The peak serum GH concentration following the combined administration of the two secretagogues was marked at 142 ± 26 mU/L for the younger subjects and 122 ± 24 mU/L in the older.

Ghigo *et al* (1994b) compared the effect of oral GHRP-6 (300 μg/kg) in a group of 8 young adults (24-32 years) with that in 8 elderly subjects (66-88 years). The peak GH response was not significantly different between the two groups (16.2 ± 5.4 μg/L *vs* 9.9 ± 2.0 μg/L, respectively). In the same study, Ghigo reported the finding that a small dose of oral arginine enhanced the GH response to GHRP-6 in the elderly but not in the young. In a further study of another group of 7 elderly subjects (65-82 years), the effect of oral GHRP-6 on GH secretion after 4 days of treatment with the peptide (300 μg/kg twice daily) was found to be maintained, with a trend towards an increase (Ghigo *et al.*, 1994c). IGF-1 levels, however, did not change after this short-term treatment. Arvat *et al* (1994) reported a lower GH response to i.v. hexarelin (2.0 μg/kg) in 8 elderly subjects (65-84 years) compared to a group of 7 young subjects (33.5 ± 11.3 μg/L *vs* 72.0 ± 7.1 μg/L, $p < 0.002$). The combined administration of hexarelin (2.0 μg/kg) plus GHRH (2.0 μg/kg) resulted in a greater GH response in both age groups, though the response in the older subjects was significantly lower than that in the younger. The administration of an i.v. infusion of arginine with the i.v. bolus of hexarelin enhanced the GH response in the elderly subjects but not the young.

Side-effects of growth hormone releasing peptides

a) Subjective

GHRPs are well tolerated in man. The most frequent adverse effect, which is infrequently observed and occurs mainly following intravenous administration, is mild transient facial flushing or sensation of warmth, lasting less than 15 minutes (Hartman *et al.*, 1992; Ghigo *et al.*, 1994a; Imbimbo *et al.*, 1994). In the case of hexarelin, this is observed more frequently with the high dose of 2.0 μg/kg, which may be also rarely accompanied by slight and transient drowsiness (Ghigo *et al.*, 1994a; Maccario *et al.*, 1995, Arvat *et al.*, 1995). No such symptoms have been reported after intranasal or oral administration of GHRPs. Intranasal administration of GHRP-2 (30 μg/kg) has been reported to cause a mildly uncomfortable sensation in the nasal mucosa (Hayashi *et al.*, 1991). Several subjects have noted that oral GHRP-6 solution had a bitter taste (Hartman *et al.*, 1992).

b) Clinical and laboratory

GHRP administration is not associated with clinically significant changes in blood pressure, pulse rate or temperature. No changes in ECG, clinical chemistry or haematology have been observed (Ilson *et al.*, 1989; Bowers *et al.*, 1990; Hartman *et al.*, 1992; Huhn *et al.*, 1993; Imbimbo *et al.*, 1994).

There are no data on the effect of chronic administration of GHRPs on body weight in humans. In animal studies, 9-day intraperitoneal or 25-day s.c. administration of GHRP-6 to immature female rats resulted in a significant increase in body weight (Bowers *et al.*, 1984). The intracerebroventricular administration of increasing doses of GHRP-6 into sated, adult male rats by Locke *et al* (1995) resulted in a nearly linear, statistically significant ($p < 0.01$) dose-response relationship between the dose of GHRP-6 and the incidence of eating. The mechanism for this behaviour was unclear and was independent of its GH-releasing property, since the mean change from baseline of plasma GH during the 60 minutes after injection was not dose-related.

The effect of GHRPs on other endogenous hormones generally and pituitary hormones specifically has been an area of much interest and great importance. The original report on the *in vitro* activity of the enkephalin analogue Tyr-DTrp-Gly-Phe-Met-NH_2 indicated that it was specific for GH release and that it had no effect on TSH, LH, FSH, PRL or ACTH (Bowers *et al.*, 1980). When Bowers and co-workers reported similar findings *in vitro* and *in vivo* for the more potent GHRP-6, it was branded a breakthrough in the development of GH secretagogues (Bowers *et al.*, 1984). Data on experimental animals by others was in keeping with Bowers's findings (Malozowski *et al.*, 1991). However, when the era of clinical studies of GHRPs started in the late 1980s, some of these findings could not be replicated in the human species.

To date, there have been no reports of a GHRP effect on LH or FSH. TSH, ACTH, PRL and cortisol, however, are affected by GHRP administration. Ilson *et al* (1989) reported a small but insignificant rise in serum PRL concentration following i.v. GHRP-6, but no change in serum TSH or ACTH concentrations. Bowers *et al* (1990) demonstrated that i.v. GHRP-6 (1.0 μg/kg) was not completely specific to the release of GH, since it resulted in a small increase in serum cortisol and PRL, though the increases were within the normal range. Intravenous administration of GHRP-1 in normal children resulted in a significant reduction in serum TSH and free T4 and a significant rise in serum cortisol, but no change in serum PRL (Laron *et al.*, 1993). Subcutaneous (3.0 μg/kg) and oral (300 or 600 μg/kg) administration of GHRP-1 resulted in a slight but insignificant rise in serum PRL and cortisol (Bowers, 1993a). Intravenous GHRP-2 (1.0 μg/kg) also resulted in a slight but insignificant rise in serum PRL and cortisol concentration, but had no effect on plasma TSH and insulin levels (Hayashi *et al.*, 1991). The administration of i.v. (1.0 or 2.0 μg/kg), s.c. (1.5 or 3.0 μg/kg), i.n. (20 μg/kg) and p.o. (40 mg) hexarelin to healthy adults resulted in a slight rise in serum PRL and cortisol concentrations, although both remained within the normal limits. Such effects on serum PRL and cortisol were not observed with the lower oral hexarelin dosage of 20 mg (Ghigo *et al.*, 1994a). In normal pubertal and prepubertal children, i.v. hexarelin (2.0 μg/kg) resulted in a significant increase in both serum cortisol and PRL concentrations, which returned to baseline values within 2 hours (Loche *et al.*, 1995a).

Section 3. Site and Mechanism of Action of Growth Hormone Releasing Peptides

The physiology of growth hormone secretion

GH secretion in man is largely controlled by the stimulatory action of GHRH and the inhibitory influence of SS (Figure 5). The pulsatile nature in which GH is secreted from the anterior pituitary could be explained on the basis of asynchronous, periodic release of GHRH and SS. A GH pulse is then expected when maximal GHRH and minimal SS secretion occurs. The hypothalamic GHRH and SS neurones reciprocally control each other through direct synaptic actions (Epelbaum *et al.*, 1989; Willoughby *et al.*, 1989), a mechanism which maintains their respective discharges out of phase. The functioning of GHRH and SS neurones is in turn regulated by a complex network of neurotransmitters, such as acetylcholine (Casanueva *et al.*, 1983) and catecholamines (Tuomisto and Mannisto, 1985), from higher centres. The central nervous system information relevant to the control of GHRH and SS, and subsequently GH release, comes from within in the form of endogenous rhythms, as well as from the outside world in the form of environmental stimuli or stresses, such as pain, physical activity and nutrient intake. GH itself exerts, through a short feedback loop, an inhibitory action on GHRH messenger ribonucleic acid (mRNA) synthesis (Chomczynski *et al.*, 1988) and a stimulatory one on SS release (Chihara *et al.*, 1981). GH also induces local synthesis of IGF-1 which inhibits subsequent GH synthesis and release by an autocrine mechanism (Yamashita and Melmed, 1986; Becker *et al.*, 1995) (Figure 5).

In addition to the above central mechanisms, multiple peripheral regulatory loops participate in the normal control of GH secretion. Thyroxine (T4) and triiodothyronine (T3) stimulate GH synthesis (Yaffe and Samuels, 1984). Glucocorticoids exert a stimulatory action upon specific corticoid-responsive elements of the GH gene (Nyborg *et al.*, 1984). However, both stimulatory (acute glucocorticoid administration) and inhibitory (chronic administration)

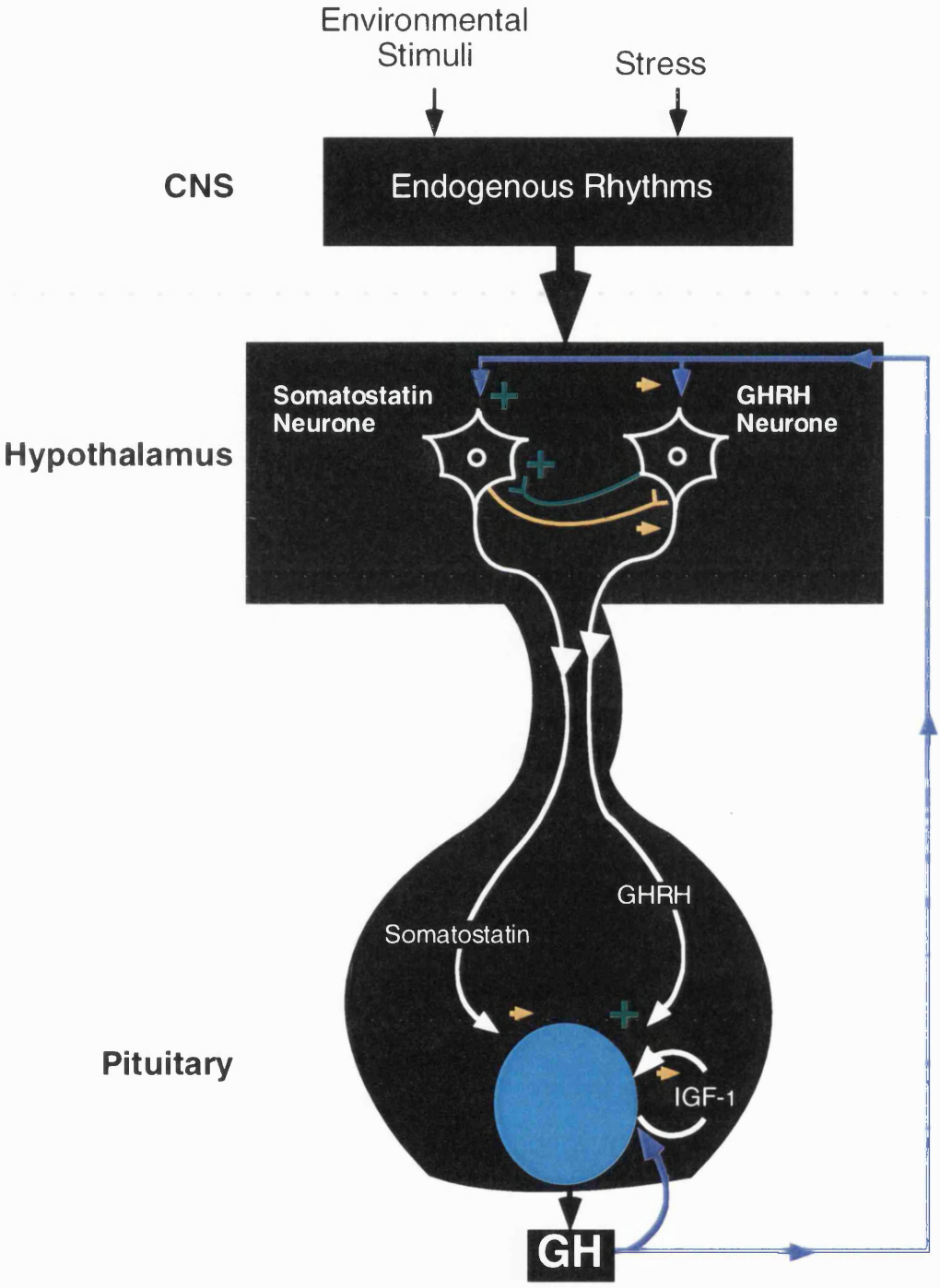


Figure 5. Scheme for central pathways involved in the control of GH secretion.

actions have been observed (Casanueva *et al.*, 1990; Frantz and Rabkin, 1964). Peripherally produced IGF-1, mainly from the liver, inhibits GH release both by direct action and by increasing SS secretion (Berelowitz *et al.*, 1981; Chomczynsky *et al.*, 1988). Sex hormones play an important role in the regulation of GH secretion, which is evident from their effect on spontaneous GH secretion at puberty (Mauras *et al.*, 1987).

GH plays an important role in regulating metabolism. In turn, GH secretion is affected by changes in the metabolic state. Hypoglycaemia is a powerful stimulus of GH secretion (Roth *et al.*, 1963). Conversely, hyperglycaemia suppresses basal GH secretion. GH has lipolytic actions, and lowering free fatty acids (FFA) causes the release of GH. Elevation of FFA, on the other hand, blocks the GH discharge induced by a variety of stimuli (Imaki *et al.*, 1985). The anabolic effects of GH are exerted through the uptake of amino acids by peripheral tissues to favour protein synthesis. An increase in blood levels of amino acids leads to a clear cut GH discharge (Knopf *et al.*, 1965).

The role of GHRPs in the physiology of GH secretion remains unknown. Much work has been carried out to elucidate their site and mode of action, which are the subject of this Section. Attempts to identify a natural ligand have so far been unsuccessful (Codd *et al.*, 1989; Bercu *et al.*, 1992; Bitar *et al.*, 1991; Pong *et al.*, 1996). However, a G protein-coupled receptor of the pituitary and arcuate ventro-medial and infundibular hypothalamus of swine and human has been cloned and has been shown to be the target of GHRPs (Howard *et al.*, 1996). On the basis of its pharmacological and molecular characterization, this G protein-coupled receptor defines a neuroendocrine pathway for the control of pulsatile GH release and supports the notion that GHRPs mimic an undiscovered hormone.

Site of action of growth hormone releasing peptides

a) Location and specificity of the growth hormone releasing peptide receptor

Early on in the search for the GHRP receptor binding studies demonstrated specific binding

of GHRPs to both rat and porcine anterior pituitary and hypothalamic membranes (Codd *et al.*, 1989; Sethumadhavan *et al.*, 1991; Veeraragavan *et al.*, 1992). Using [^{3}H]GHRP-6, a tritiated ligand of GHRP-6, and [^{125}I]Tyr-Ala-GHRP-6, an iodinated octapeptide analogue of GHRP-6 with unaltered biological potency, binding to pituitary and hypothalamic membranes of these species was found to be specific, saturable, reversible and time-, temperature-, pH- and concentration-dependent (Codd *et al.*, 1989; Sethumadhavan *et al.*, 1991; Veeraragavan *et al.*, 1992). (D-Lys^{3})GHRP, a known GHRP antagonist, was found to be a potent inhibitor of GHRP binding to these sites. These properties fulfilled the major requirements of a true hormone-receptor interaction.

Muccioli *et al* (1995) studied the binding of [^{125}I]Tyr-Ala-hexarelin to membranes from the pituitary gland and various regions of the human brain (hypothalamus, cerebral cortex, cerebellum and choroid plexus) derived from autopsy specimens. Among the various tissues studied, the hypothalamus and the pituitary gland showed the highest specific binding. Clearly detectable specific binding was also observed in the choroid plexus and the cerebral cortex, whereas it was negligible in the cerebellum. The binding of [^{125}I]Tyr-Ala-hexarelin to hypothalamic tissue was inhibited in a dose-dependent manner by both hexarelin and GHRH, though hexarelin was more effective than GHRH. In contrast, no competition was displayed by human GH or human PRL. Scatchard analysis of the binding revealed the presence of a single class of saturable binding sites with high affinity for hexarelin ligand. Thus, the study by Muccioli *et al* (1995) provided strong preliminary evidence that the human brain and pituitary gland contain significant amount of hexarelin receptors.

The recent work of Howard *et al* (1996) confirmed the presence of human GHRP receptor mRNA in the pituitary gland, hippocampus, arcuate-ventromedial hypothalamus and infundibular hypothalamus. GHRP receptor mRNA could not be detected in whole brain, liver, spleen, placenta and kidney.

As demonstrated by Blake and Smith (1991), the GHRP receptor is distinct from that of GHRH. In their study, pituitary cells were perifused separately with GHRP-6 and GHRH. The continuous exposure of the cells to either secretagogue produced a time-dependent loss

of specific secretagogue responsiveness in the perifusion system, consistent with receptor desensitisation rather than complete GH depletion. Challenge by the alternative secretagogue immediately resulted in a secondary release of GH. Similar findings were reported by Cheng *et al* (1989). These results suggest that GHRP-6 acts through a receptor site distinct from that of GHRH, a fact that is further supported by the following findings: (i) kinetics of desensitisation are different for GHRP-6 and GHRH (Blake and Smith, 1991); (ii) the two secretagogues employ different binding sites (Codd *et al.*, 1989); (iii) specific inhibitors of each secretagogue have been identified (Cheng *et al.*, 1989); (iv) each secretagogue activates different signal transduction pathways (Cheng *et al.*, 1989); (v) the two secretagogues in combination produce larger GH responses than either agent alone (Sartor *et al.*, 1985); and (vi) the two secretagogues produce different patterns of GH secretion (McCormick *et al.*, 1985).

The GHRP receptor sites, at least those involved in GH release, have also been shown to be distinct from those of other neuropeptides. Goth *et al* (1992) demonstrated that GHRP and PACAP (pituitary adenylate cyclase-activating polypeptide) acted via different receptors and that they had differential effects on the number of cells secreting GH and the amount of GH secreted per cell. The possibility that GHRPs act through opiate receptors was investigated *in vitro* by Codd *et al* (1988 & 1990) and Cheng *et al* (1989). Binding studies confirmed that the GH-releasing action of GHRP-6 did not involve opioid receptor binding (Codd *et al.*, 1988 & 1990). Naloxone, an opiate antagonist, had no effect on GHRP-6-induced GH release in rat pituitary cell culture (Cheng *et al.*, 1989). Korbonits *et al* (1995) showed that naloxone did not affect hexarelin-induced GH release in a group of healthy adult humans. While these data dissociate the opioidergic properties of GHRPs from their GH-releasing effects, GHRPs may well have other opiate-like activities, such as the transient and weak analgesic effect following subcutaneous administration (Codd and Walker, unpublished) and the promotion of stage II sleep (Frieboes *et al.*, 1995).

b) Hypothalamic or pituitary action ?

The prime site of action of GHRPs remains controversial. One site of action must be within

the pituitary gland since GHRPs can induce GH release from pituitary cells *in vitro* (Bowers *et al.*, 1984; Badger *et al.*, 1984; Sartor *et al.*, 1985; Edwards *et al.*, 1989; Cheng *et al.*, 1989; Bowers *et al.*, 1991b; Goth *et al.*, 1992; Akman *et al.*, 1993; Wu *et al.*, 1996). *In vivo* evidence for a pituitary site of action is provided by the ability of GHRP to release GH in conditions of functional hypothalamopituitary disconnection (Mallo *et al.*, 1993; Fletcher *et al.*, 1994; Popovic *et al.*, 1995), though the lack of consensus on diagnostic criteria of these conditions in man precludes from making a definitive statement. A pituitary site of action is further suggested by the presence of specific GHRP binding sites on anterior pituitary membranes, as discussed above.

Despite the clear evidence for pituitary action, researchers have argued for an additional hypothalamic site of action (Clark *et al.*, 1989). Action on the hypothalamus is suggested by the presence of specific hypothalamic binding sites (see above), more easily identifiable than those on the pituitary. The synergistic GH-releasing action of GHRP and GHRH *in vivo* (Bowers *et al.*, 1990; Bowers *et al.*, 1991b; Bowers, 1993a) and its absence *in vitro* (Sartor *et al.*, 1985; Bowers *et al.*, 1991b) argue for an interaction of the two secretagogues at a site other than the pituitary with the possibility of an, as yet, unknown factor being released from the hypothalamus.

Direct evidence for a hypothalamic site of action is provided by the work of Dickson *et al* (1993, 1995a & 1995b) who found that systemic or central administration of GHRP activated hypothalamic arcuate neurones in Wistar rats, GH-deficient dwarf (*dw/dw*) rats and GH-deficient little (*lit/lit*) mice. This subpopulation of hypothalamic neurones was found to have strongly increased *fos* (the protein product of the immediate early gene, *c-fos*, which is induced in many neuronal systems following their activation) expression, in response to GHRP administration. Extracellular recordings from putative neurosecretory neurones, most likely GHRH neurones, in the arcuate nucleus showed that GHRP also stimulated the firing of neurones in this area (Dickson *et al.*, 1993 & 1995a).

The work of Fairhall *et al* (1995) further supports a hypothalamic site of action of GHRPs. Central (intracerebroventricular) administration of GHRP-6 to anaesthetized guinea pigs led

to a large GH release. The same GH response required in excess of 20-fold more GHRP-6 when given intravenously, indicating the sensitivity of the hypothalamic (central) response and the likelihood that the response was not mediated through action at the pituitary. Furthermore, central SS administration blocked the GH release in response to central GHRP-6 injection, indicating interaction at the hypothalamic level. This effect was not due to SS leaking out to the pituitary to block GH output directly since the basal GH release was unaffected by SS injections and the pituitary was still able to respond to a peripheral challenge with GHRH to release GH.

Finally, the ability of hexarelin to increase GHRH levels in the hypophysial portal blood of conscious sheep associated in time with the stimulation of GH secretion lends further evidence for a hypothalamic site of action of GHRPs (Guillaume *et al.*, 1994).

Mechanism and regulation of growth hormone releasing peptide action

It is clear from the above account that GHRPs act on both the hypothalamus and the pituitary, though the relative importance of these two sites of action is still debated. Much work has been done to elucidate this issue, the mechanism of action of GHRPs, the relationship between the actions of GHRPs, GHRH and SS as well as the interaction of GHRPs with other modulators of GH release (Clark *et al.*, 1989; Cheng *et al.*, 1989; Edwards *et al.*, 1989; Malozowski *et al.*, 1991; Bercu *et al.*, 1992; Mallo *et al.*, 1993; Guillaume *et al.*, 1994; Conley *et al.*, 1995; Arvat *et al.*, 1995; Maccario *et al.*, 1995; Dickson 1995a & 1995b; Fairhall *et al.*, 1995), but no consensus in the interpretation of these results has been reached yet.

a) Growth hormone releasing peptide and growth hormone releasing hormone

Since GHRPs can directly stimulate GH release from the pituitary *in vitro* (Bowers *et al.*,

1984; Badger *et al.*, 1984; Sartor *et al.*, 1985; Bowers *et al.*, 1991b; Goth *et al.*, 1992; Akman *et al.*, 1993; Wu *et al.*, 1996) they can clearly function independently of GHRH. The greater maximal GH response to GHRPs compared to GHRH and their nonparallel dose-response curves reported by Malozowski *et al* (1991) supports the notion that GHRPs work by a mechanism other than release of endogenous GHRH, and probably independently of it. In addition, most studies suggest that GHRPs do not act as agonists on the GHRH receptor (Codd *et al.*, 1989; Bitar *et al.*, 1991; Sethumadhavan *et al.*, 1991; Blake and Smith, 1991; Goth *et al.*, 1992; Dickson *et al.*, 1995b).

However, there are data that show that the GH-releasing action of GHRPs is dependent on GHRH. Bercu *et al* (1992) found that the GH-releasing activity of GHRP-6 was almost completely suppressed in rats preimmunised passively against endogenous GHRH. Similar findings have been reported for GHRP-6 (Clark *et al.*, 1989; Bowers *et al.*, 1992) and hexarelin (Conley *et al.*, 1995). In the study by Bercu *et al* (1992), GHRP-6 activity was markedly stimulated by relatively low GHRH agonist activity. These data suggest that GHRH is "permissive" to the action of GHRP in that at least minimal activation of pituitary GHRH receptors is required for full expression of GHRP. Furthermore, the data from Jansson *et al* (1986a) and Dickson *et al* (1995b) indicated that normal functioning of GHRH was essential for GH release by GHRP. GHRP-6 was unable to induce GH release in the *lit/lit* mouse (Jansson *et al.*, 1986a), which has a mutation in the extracellular binding domain of the GHRH receptor (Lin *et al.*, 1993) and therefore does not respond to GHRH administration (Jansson *et al.*, 1986b), but was able to activate a subpopulation of arcuate neurones, indicating intact hypothalamic function (Dickson *et al.*, 1995b). The inference from these findings was that the pituitary actions of GHRP-6 were dependent upon the existence of a functional GHRH receptor. The finding that hexarelin increased GHRH levels in portal blood in time with its GH-induced release (Guillaume *et al.*, 1994) is another piece of evidence linking GHRP function with GHRH. Fairhall *et al* (1995) linked these findings with his own, namely, the hypothalamic effects of GHRP-6 (Fairhall *et al.*, 1995 - see above), and concluded that one central effect of GHRPs is to activate GHRH neurones either directly or indirectly via other neuronal targets.

An intriguing phenomenon of GHRPs and GHRH is their synergistic GH-releasing action, i.e. the amount of GH secreted following their concomitant administration is greater than the arithmetic sum of the GH secreted following their isolated administration. Except for results of one *in vitro* study (Cheng *et al.*, 1989) when synergism occurred, the combined effects of GHRP plus GHRH *in vitro* have been either additive or minimally synergistic (Sartor *et al.*, 1985; Bowers *et al.*, 1991b) and insufficient in magnitude to compare with the much greater synergism observed *in vivo* (Bowers *et al.*, 1990; Bowers *et al.*, 1991b; Muruais *et al.*, 1993; Peñalva *et al.*, 1993b; Bowers, 1993a; Leal-Cerro *et al.*, 1994; Arvat *et al.*, 1994; Micic *et al.*, 1995; Pihoker *et al.*, 1995a). Because of these results, the synergistic effects of GHRP plus GHRH have been postulated to occur via a hypothalamic anatomic site of action, though the exact mechanism by which this happens remains unclear. GHRPs do not inhibit hypothalamic SS release but can induce GHRH release (Guillaume *et al.*, 1994). The latter observation is unlikely to account for the synergistic action of the two secretagogues because even high doses of GHRH combined with GHRP *in vitro* fail to produce the massive GH release observed *in vivo* (Bowers *et al.*, 1991b), and the *in vivo* synergistic effect still occurs when maximal doses of GHRH and GHRP are used (Peñalva *et al.*, 1993a and 1993b). In any case, GHRH release cannot be the sole hypothalamic mechanism of GHRP action since the latter continues to release GH after GHRH responsiveness has been lost (McCormick *et al.*, 1985; Malozowski *et al.*, 1991; Robinson *et al.*, 1992). An interesting hypothesis, which is still much debated and will be discussed later in this Section, is that GHRPs may inhibit the pituitary action of SS. Another hypothesis, put forward by the originator of GHRP-6, is that GHRPs release an, as yet, unidentified hypothalamic factor (U-factor) which interacts in combination with GHRH on the pituitary to release GH synergistically. The pituitary action of the U-factor depends on the presence of GHRH, because GHRH antiserum inhibits the synergistic GH response to GHRP plus GHRH (Bowers *et al.*, 1991b). The effect of the hypothetical U-factor itself on GH release is considered to be minimal, because GHRPs would presumably still release U-factor despite pretreatment with GHRH antiserum and yet GH release is low.

b) Growth hormone releasing peptide and somatostatin

The early *in vitro* and *in vivo* studies showed that SS was able to attenuate (and even inhibit) GHRP-induced GH release (Bowers *et al.*, 1984). Many years later, SS inhibition of GHRP-induced GH release was reported by others (Blake and Smith, 1991; Goth *et al.*, 1992; Renner *et al.*, 1994; Peñalva *et al.*, 1993b; Arvat *et al.*, 1995). Interestingly, while SS inhibited the GH response to GHRP-6 alone or GHRH alone, it was unable to abolish it completely following their combined administration (Blake and Smith, 1991).

The mechanism by which SS inhibits GHRP-induced GH release remains unclear. Some interaction between SS and GHRP must occur at the pituitary level, as evident from the inhibition of GH release *in vitro*. SS and GHRP do not compete for the same receptor sites as demonstrated by the lack of SS binding to the GHRP receptor identified by Howard *et al* (1996). Since the GHRP-induced GH release can be inhibited by SS and the inhibitory potency of SS can be reduced by GHRP (Bowers *et al.*, 1994), it is possible that GHRP and SS could act as functional antagonists at the pituitary gland. It is well established that SS inhibits GH release by a cAMP-independent mechanism of action, leading to hyperpolarisation of the somatotroph cell membrane (Koch *et al.*, 1988). In contrast, GHRP depolarises somatotroph cell membranes (Pong *et al.*, 1991) and results in Ca^{2+} entry into the cell (see 'Signal transduction' below), a process which is blocked by SS. These opposite actions on membrane potential may form the basis for their functional antagonism.

GHRP and SS also interact at hypothalamic level. Clark *et al* (1989) argued that the elevated baseline serum GH concentrations during continuous i.v. GHRP-6 infusions could be explained on the basis of decreased endogenous SS tone. Dickson *et al* (1995a) found that, although the effect of intravenous administration of GHRP-6 on the electrical activity of arcuate nucleus neurones was predominantly excitatory for putative neuroendocrine cells, it was inhibitory for the remaining unidentified cells. The latter were arcuate nucleus neurones which did not project to the median eminence and would therefore do for somatostatinergic neurones. If this were the case, then GHRP may be acting at the hypothalamic level to inhibit somatostatinergic neuronal activity and hence SS release. However, there is evidence that contradicts this hypothesis. Guillaume *et al* (1994) showed that SS secretion into the hypophysial portal blood remained unaltered following hexarelin administration in sheep. The

potentiation of the GH response to GHRP-6 in rats by pretreatment with SS antiserum argues against the peptide having an inhibitory effect on SS release (Bowers *et al.*, 1991b). The strongest evidence for the hypothalamic interaction of GHRP and SS was provided by Fairhall *et al* (1995), who showed that pretreatment of guinea pigs with an intracerebroventricular injection of a long acting SS analogue blocked the GH release in response to central GHRP-6 administration. Fairhall *et al* (1995) concluded that the hypothalamic mechanism by which GHRP-6 released GH was sensitive to inhibition by SS. Since GHRPs act at the hypothalamus to stimulate GHRH neurones and GHRH release (see above), and since GHRH neurones are known to co-localise SS receptors (Epelbaum *et al.*, 1989) which could regulate GHRH release in response to SS, it was hypothesized that GHRPs and SS were functional antagonists at central SS receptors inhibitory to GHRH release.

c) Growth hormone releasing peptide and other modulators of growth hormone secretion

Edwards *et al* (1989) showed that the GH response to GHRP-6 in *in vivo* and *in vitro* models of hypothyroidism was reduced compared to euthyroid controls, though no conclusions about the mechanism for this finding could be drawn from their studies. Mallo *et al* (1993) studied the influence of glucocorticoids, sex hormones (oestradiol and testosterone administered separately) and FFA on the GH response to GHRP-6 in rats. Except for the sex hormone treatment group, where gonadectomised animals were used, the studies were conducted on intact rats. Chronic (15 days) dexamethasone treatment markedly decreased the GH response to GHRP-6, while both gonadal steroids (given every 3 days for 15 days) enhanced the response. Increasing plasma FFA by means of an acute i.v. intralipid infusion 30 minutes before GHRP-6 administration resulted in inhibition of the GH response to the peptide. Similarly, Maccario *et al* (1995) found that an oral glucose load (100 gm) or an i.v. infusion of a 10% lipid solution blunted, but did not abolish, the GH response to hexarelin in healthy adult humans. Insulin-induced hypoglycaemia potentiated the GH releasing effect of GHRP-6 in humans (Peñalva *et al.*, 1993b). These data suggest that GHRP-induced GH secretion can be modulated by various hormonal and metabolic factors, some of which (e.g. FFA and glucose) are thought to exert their inhibitory action on GH secretion by increasing

hypothalamic SS release.

Other researchers have investigated the effect of neuroactive substances on the GH-releasing activity of GHRPs. Noradrenergic and cholinergic pathways acting through different receptor types play an important role in the regulation of GH secretion and are thought to do so by modulating SS neurones (Dieguez *et al.*, 1988; Ghigo 1992 p.103). So far, five studies documenting such interactions have been published (Peñalva *et al.*, 1993a; Peñalva *et al.*, 1993b; Muruais *et al.*, 1993; Arvat *et al.*, 1995; Arvat *et al.*, 1996). The findings of these studies are summarised in Table 8.

Table 8. Effect of neuroactive substances on the GH response to GHRPs.

Type of study	Neuroactive substance	Effect on GHRP-induced GH secretion	Reference
in vivo (humans)	Propranolol (β-adrenergic antagonist)	No significant effect (GHRP-6)	Peñalva *et al.*, 1993a
in vivo (humans)	Prazosin (α-1 adrenergic antagonist)	Significant increase (GHRP-6)	Peñalva *et al.*, 1993a
in vivo (humans)	Clonidine (α-2 adrenergic agonist)	No significant effect (GHRP-6)	Peñalva *et al.*, 1993a
in vivo (humans)	Atropine (cholinergic antagonist)	Complete suppression (GHRP-6)	Peñalva *et al.*, 1993b
in vivo (humans)	Pyridostigmine (cholinergic agonist)	Slight but significant increase (GHRP-6)	Peñalva *et al.*, 1993b
in vivo (dogs)	Atropine (cholinergic antagonist)	Complete suppression (GHRP-6)	Muruais *et al.*, 1993
in vivo (dogs)	Pyridostigmine (cholinergic agonist)	Significant increase (GHRP-6)	Muruais *et al.*, 1993
in vivo (dogs)	Prazosin (α-1 adrenergic antagonist)	Significant decrease (GHRP-6)	Muruais *et al.*, 1993
in vivo (dogs)	Clonidine (α-2 adrenergic agonist)	No significant effect (GHRP-6)	Muruais *et al.*, 1993
in vivo (dogs)	Methoxamine (α-1 adrenergic agonist)	No significant effect (GHRP-6)	Muruais *et al.*, 1993
in vivo (humans)	Pirenzepine (muscarinic antagonist)	Significant reduction (hexarelin)	Arvat *et al.*, 1995
in vivo (humans)	Pyridostigmine (cholinergic agonist)	No significant effect (hexarelin)	Arvat *et al.*, 1995
in vivo (humans)	Arginine (an amino acid)	No significant effect (hexarelin)	Arvat *et al.*, 1995
in vivo (humans)	Atenolol (β-adrenergic antagonist)	No significant effect (hexarelin)	Arvat *et al.*, 1996
in vivo (humans)	Salbutamol (β-adrenergic agonist)	Significant reduction (hexarelin)	Arvat *et al.*, 1996

Cellular effects of growth hormone releasing peptides

a) Nature of the receptor

The initial binding assays using [^{3}H]GHRP-6 and ^{125}I-labelled analogues of GHRPs failed to correlate binding specificity with GH-secretory activity of peptide and non-peptide secretagogues. The binding was also of relatively low affinity and high capacity (Pong *et al.*, 1996). An alternative ligand, ^{35}S-labelled MK-0677, was sought and developed by Pong *et al* (1996). This ligand was shown to have saturable and high affinity binding to a single class of sites in porcine and rat anterior pituitary membranes. Competitive binding studies showed that relative binding affinity was predictive of the GH-secretory activity, with the most potent agonists having the highest affinities for pituitary receptor sites. Thus a new receptor site was identified to which peptide (GHRP-6 and hexarelin) and non-peptide (L-692,585 and L-692,429) secretagogues could bind. In addition, Pong *et al* (1996) found that the stable guanylyl triphosphate (GTP) analogue guanylyl-imidodiphosphate (GMP-PNP) was a potent inhibitor of [^{35}S]MK-0677 binding while the nucleotide adenosine triphosphate(ATP)-γ-S was ineffective, and that the specific binding of [^{35}S]MK-0677 was Mg^{2+} dependent. The latter two findings indicated that this new receptor was likely to be G-protein linked. Other GHRP studies were also consistent with activation of G-protein pathways coupled to potassium channels (Pong *et al.*, 1993) and phospholipase C (Cheng *et al.*, 1991), which are involved in the process leading to the elevation of intracellular Ca^{2+} in somatotrophs and, consequently, GH release. In 1996, Howard and co-workers cloned a heterotrimeric GTP-binding protein-coupled receptor and showed it to be the target of the GHRPs (Howard *et al.*, 1996).

b) Signal transduction

The precise intracellular mechanism by which GHRPs stimulate secretion of GHRPs has not been deciphered. Unlike GHRH, which is thought to act primarily through the stimulatory G-protein-adenyl cyclase-cAMP pathway (Frohman and Jansson, 1986; Narayanan *et al.*, 1989; Mason *et al.*, 1993), GHRPs do not stimulate cAMP production (Cheng *et al.*, 1991; Akman *et al.*, 1993). However, GHRP-6 has been shown to stimulate phosphatidylinositol

turnover in a dose-dependent manner in human pituitary somatotroph cell (Lei *et al.*, 1995) and rat anterior pituitary cells (Mau *et al.*, 1995). Increased phosphatidylinositol turnover in these studies was accompanied by a dose-dependent release of GH.

Phosphatidylinositol is a second messenger system which leads to activation of protein kinase C and mobilisation of intracellular Ca^{2+}. Receptor-mediated hydrolysis of membrane phosphatidylinositol results in the release of diacylglycerol and inositol-1,4,5-triphosphate. The former directly activates protein kinase C while the latter mobilises intracellular Ca^{2+} (Nishizuka, 1988; Berridge and Irvine, 1989). Protein Kinase C also stimulates Ca^{2+} entry into the cell by phosphorylation and activation of long lasting (L)-type-Ca^{2+} channels (Nastainczyk *et al.*, 1987). Since calcium is one of the main second messengers involved in stimulus-secretion processes (Stojilkovic and Catt, 1992), the elevation of intracellular Ca^{2+} concentration due to effects of both inositol-1,4,5-triphosphate and protein kinase C most probably induce exocytosis of GH.

There is indirect evidence that the GH releasing action of GHRP-6 is partially (~70%) mediated through the activation of protein kinase C (Cheng *et al.*, 1991). This was based on the following observations: (i) protein kinase C activators mimicked the effects of GHRP-6 on GH release and synergised with GHRH to further increase GH secretion and intracellular cAMP accumulation; (ii) extracellularly added phospholipase C, which generates free endogenous diacylglycerol and in turn activates protein kinase C, stimulated GH release in a dose-dependent manner and potentiated the effect of GHRH in a similar manner to GHRP-6; and (iii) the effect of GHRP-6 on GH release was inhibited by protein kinase C inhibitors. Interestingly, GHRP-6 was still able to stimulate GH release from protein kinase C-depleted cells, although to much smaller extent than in control cells. This suggested that the predominant action of GHRP-6 on GH release was mediated through protein kinase C and that the rest was independent of it.

The effects of GHRPs on intracellular Ca^{2+} have also been studied. Herrington and Hill (1994) showed that GHRP-6 elevated intracellular Ca^{2+} in rat somatotrophs in two distinct phases. The first was rapid and transient, lasting 45-60 seconds, and resulted from the release

of Ca^{2+} from internal stores, since it occurred under conditions which prevented entry of extracellular Ca^{2+}. This rise in intracellular Ca^{2+} activated voltage-independent K^+ channels, transiently hyperpolarising the somatotroph. The transient phase was followed by a sustained elevation in intracellular Ca^{2+} which lasted several minutes and persisted after the removal of the peptide. This second phase resulted from Ca^{2+} entry, probably through voltage-dependent Ca^{2+} channels, and may be caused by a long-lasting membrane depolarisation. This biphasic Ca^{2+} response to GHRP-6 in rat pituitary somatotrophs was also reported by Bresson-Bepoldin and Dufy-Barbe (1994). The first phase of the response, characterised by a rapid sharp increase in intracellular Ca^{2+} concentration, was found to involve mobilisation of inositol-1,4,5-triphosphate sensitive intracellular Ca^{2+} stores. The second phase, characterised by a prolonged plateau phase of elevated intracellular Ca^{2+} concentration on which oscillations were superimposed, was protein kinase C-dependent and resulted from a Ca^{2+} influx. Bresson-Bepoldin and Dufy-Barbe (1994) also found that the two phases of the response could be triggered independently, thus indicating that they rely on distinct transduction mechanisms.

Akman *et al* (1993) found that treatment of rat anterior pituitary cells with GHRP-1 resulted in a rapid and sustained increase in intracellular Ca^{2+} concentration, which was likely to have occurred through the influx of Ca^{2+} via voltage-dependent Ca^{2+} channels. The findings of Akman *et al* (1993) were consistent with two independent pathways involved in the regulation of GH release: a protein kinase C pathway and a separate intracellular Ca^{2+} concentration mediated pathway stimulated by GHRP-1. Thus it seems that GHRP-6 and GHRP-1 have similar, if not identical, signal transduction pathways.

Chen and Clarke (1995) investigated the modification by GHRP-2 of membrane Ca^{2+} currents and the resultant changes in intracellular free Ca^{2+} in cultured ovine somatotrophs. GHRP-2 was found to increase both L-type and T (transient)-type Ca^{2+} currents, leading to an increase in intracellular Ca^{2+} concentration. However, the GHRP-2 receptor and signal transduction pathway seems to be different to that of GHRP-1 and GHRP-6 (Wu *et al.*, 1994 & 1996). Unlike the latter two, GHRP-2 was found to increase intracellular cAMP levels in ovine somatotrophs, probably through activation of adenyl cyclase (Wu *et al.* 1996), and its action

on the pituitary was blocked by specific GHRH receptor antagonists (Wu *et al.*, 1994). The modulation of Ca^{2+} currents by GHRP-2 observed by Chen and Clarke (1995) may therefore be mediated by a different intracellular signal to that of GHRP-1 and GHRP-6. Interestingly, the work of Wu *et al* (1996) showed that, while GHRP-2 led to an increase in cAMP levels in sheep somatotrophs, it failed to do so in rat pituitary cells, indicating species differences in the response of pituitary somatotrophs to the GHRP. This may be due to different subtypes of GHRP receptor in rat and sheep. The possibility that the GHRP receptor may have species variation was raised following the earlier *in vitro* binding studies in porcine and rat pituitary and hypothalamic membranes (Sethumadhavan *et al.*, 1991; Veeraragavan *et al.*, 1992).

c) Effect on growth hormone gene expression

There is only one published report on the GHRPs' ability to stimulate GH mRNA expression (Locatelli *et al.*, 1994). Pups were passively immunised with GHRH antibodies on days 1, 2, 4, 6, 8 and 10 of life. Control-treated rats received an equal volume of normal rabbit serum. Beginning on day 6 or 8 and up to 10 days of life, pups were given twice daily s.c. GHRP-6 or physiological saline. Twelve hours after the last administration of GHRP-6 pups were killed and the pituitary gland was quickly dissected for determination of GH gene expression. Passive immunisation with GHRH antibodies significantly reduced pituitary mRNA expression, which was fully restored to control levels by the 5 day treatment (6-10 days of life) with GHRP-6. The 3-day GHRP-6 treatment only partially restored GH mRNA expression. GHRP-6 treatment did not stimulate GH mRNA expression in the normal rabbit serum-treated rats. The latter finding may be explained on the basis that GHRP-6 is able to stimulate GH mRNA transcription only when the GHRH-dependent GH gene expression is defective. Thus, GHRP-6 stimulation would not be revealed if GH gene expression is already maximally stimulated under the physiological influence of GHRH. The ability of GHRP-6 to take over the function of GHRH when the latter is defective stresses the potential for GHRP-6 or its analogues in the treatment of GHRH-deficient states.

Soto *et al* (1995) investigated the regulation of pituitary-specific transcription factor-1 (Pit-1) mRNA levels in primary monolayer cultures of rat anterior pituitary cells. In addition to direct

activators of second messenger signalling systems, Soto *et al* (1995) studied the effects of different hormones known to be involved in the regulation of somatotroph cell function. Amongst those studied, GHRH was found to increase Pit-1 mRNA levels in a dose- and time-dependent manner, while GHRP-6 had no effect on Pit-1 mRNA levels, suggesting that GHRPs act primarily to increase GH secretion with little effect on GH synthesis. However, considering that GHRP-6 is much more potent *in vivo* than *in vitro* in terms of GH release, this study could not rule out the possibility that *in vivo* GHRP-6, by acting both at the hypothalamus and pituitary levels, may regulate Pit-1 gene expression.

Section 4. Studies of Growth Hormone Releasing Peptides in Disease States

The clinical era of GHRP studies started in 1988. In addition to studies of normal subjects eluded to in previous Sections, a number of studies of the effects of GHRPs in various disease states and conditions associated with abnormal GH secretion were carried out. These studies were important not only because they allowed documentation of the GH response to GHRPs in pathological states but also because they shed light on the interactions of GHRPs with other hormones and gave further insight into the mechanism of action of GHRPs.

Growth hormone deficiency states

GH deficiency states, including hypothalamopituitray disconnection, in adults and children, are covered in Section 5 of this Chapter under the heading 'Diagnostic tests'.

Acromegaly

Several pathophysiological mechanisms produce the clinical syndrome of acromegaly, with a primary pituitary tumour being the most common cause (Melmed, 1991). GH secretion in acromegaly is characterised by increased GH release, with a concomitant increase in plasma IGF-1 concentration as well as varied and sometimes paradoxical responses to a variety of hormonal and metabolic stimuli, such as TRH, gonadotrophin releasing hormone (GnRH), dopamine, and glucose. With these facts in mind, researchers became interested in investigating the GH response to GHRPs in acromegalic patients, and determining its usefulness in characterising the mechanism(s) of GH secretion in acromegaly.

Alster *et al* (1993) studied the GH responses to i.v. GHRH, GHRP-6 and TRH in 11 patients

with active acromegaly. All subjects responded to GHRH and GHRP-6, and 9 out of 10 responded to TRH. There was good correlation between the basal and peak GH values for GHRH, GHRP-6 and TRH stimulation. The time courses to the peak GH responses to GHRH and GHRP-6 were similar. When the GH response to GHRP-6 was compared with the GH response to GHRH and TRH, a significant correlation was seen with the latter but not with former, suggesting that GHRPs and TRH may act through similar mechanisms to release GH in acromegaly. The authors concluded that GHRPs were unlikely to be of diagnostic value in acromegalics since their GH responses to GHRP-6 were qualitatively normal and the time course as well as the heterogeneity of the GH response were similar to the responses observed in normal healthy subjects.

In a similar study, Popovic *et al* (1994) investigated the effect of i.v. GHRH, GHRP-6 and GHRH plus GHRP-6 in 11 patients with active acromegaly and 12 normal subjects. Acromegalic patients responded in a similar pattern to normals to stimulation with GHRH, GHRP-6 and GHRH plus GHRP-6, though the peak GH responses were greater in magnitude in the acromegalic patients. When eliminating the high basal GH levels of acromegalic patients by analysing data as area under the curve (AUC), the general patterns of the GH discharge in acromegalic subjects were practically identical, although enhanced, with respect to that in the normal control subjects. One major difference observed was the absence of synergistic action in acromegalic subjects following the combined administration of GHRH plus GHRP-6. Since the serum GH in acromegaly derives from neoplastic tissue which is not under hypothalamic control, this finding suggested that for the potentiating effect of both compounds to occur a normal hypothalamopituitary connection must be present. This is supported by studies of GHRPs in hypothalamopituitary disconnection syndromes (see Section 5, 'Diagnostic studies').

In contrast to the above findings by Popovic *et al* (1994), Hanew *et al* (1994) documented synergism between GHRP and GHRH in 10 out of 11 subjects with active acromegaly who had responded to isolated GHRP administration. All 11 subjects had a large GH response to isolated administration of GHRH and TRH. The mean time to peak GH was similar following GHRP and TRH, and was significantly shorter than that following GHRH. There were no

correlations in the maximum GH increments between GHRP and TRH or between GHRP and GHRH. The response pattern to GHRP was different from GHRH but was similar to that of TRH suggesting that, in acromegaly, the mode of action of GHRP is similar to TRH but different to GHRH.

Ciccarelli *et al* (1996) showed that the GH response to hexarelin in acromegalic patients was similar to that in normal subjects. They also showed that the prolactin-releasing effect of the peptide was preserved in patients with active acromegaly.

Hyperprolactinaemia

The actions of GHRPs in hyperprolactinaemic subjects have been reported by one group only (Ciccarelli *et al.*, 1996). In addition, there are no data on the effects of GHRPs on prolactin secretion *in vitro*. Ciccarelli *et al* (1996) showed that the GH-releasing effect of hexarelin was blunted and that its prolactin-releasing effect was absent in patients with pathological hyperprolactinaemia, defined as having a mean prolactin concentration of greater than 1000 mU/L in subjects who had a microadenoma or empty sella syndrome.

Glucocorticoid excess / Cushing syndrome

As discussed in chapter 3, glucocorticoids have long been known to interfere with normal somatic growth in laboratory animals and in man. Patients with Cushing syndrome and normal subjects who are given supraphysiological doses of glucocorticoids have blunted GH responses to physiological and pharmacological stimuli (Demura *et al.*, 1972; Giustina *et al.*, 1990, 1991).

Giustina *et al* (1995) studied the GH responses to hexarelin, GHRH and hexarelin plus GHRH in 7 adults with chronic glucocorticoid excess. Six subjects were undergoing long-term (> 6 months) immunosuppressive glucocorticoid treatment for non-endocrine diseases and 1 had endogenous hypercortisolism due to an adrenal tumour. Whilst the GH response to GHRH

was suppressed in these subjects, the response to hexarelin was no different from that seen in normal subjects. However, the peak response to the combined administration of hexarelin plus GHRH was significantly lower than that observed in normal subjects, though it remained synergistic. This study showed that the GH-releasing activity of hexarelin was not affected by a state of chronic glucocorticoid excess. With recent studies showing that the inhibitory effect of glucocorticoids on GH secretion may be mediated by enhanced SS tone (Giustina and Wehrenberg, 1992) the findings by Giustina *et al* (1995) suggests that GHRPs act, at least in part, by counteracting the effect of SS on the somatotroph.

In contrast to the above findings, Leal-Cerro *et al* (1994) found that the GH responses to GHRP-6, GHRH and GHRP-6 plus GHRH were severely blunted in 10 patients with untreated Cushing syndrome. Moreover, the synergistic action of GHRP-6 plus GHRH was absent in the group of patients studied. In fact, only 1 out of the 10 subjects had a GH response of >20 mU/L following the combined administration of the two secretagogues. The differences observed between the two studies may be related to the underlying aetiology of the hypercortisolaemic state, namely, exogenous administration of cortisol (Giustina *et al.*, 1995) *vs* pituitary-dependent Cushing syndrome (Leal-Cerro *et al.*, 1994). However, in each study there was one case of Cushing syndrome secondary to an adrenal tumour.

Hyperthyroidism

Thyroid hormones influence GH synthesis and secretion by the somatotroph both *in vivo* and *in vitro*, although their precise site of action remains unknown (Valcavi *et al.*, 1992). In patients with hyperthyroidism, an altered GH response to several pharmacological stimuli, including GHRH, has been found (Valcavi *et al.*, 1992; Burgess *et al.*, 1966; Valcavi *et al.*, 1993).

Ramos-Dias (1996) studied a group of 9 women with hyperthyroidism due to Graves' disease. All had symptoms and signs of thyrotoxicosis and none was taking any medication for at least three months before the study. Each subject was given i.v. GHRP-6, GHRH and GHRH plus GHRP-6 on three separate occasions. The GH response to GHRH and GHRH plus GHRP-6

was suppressed in hyperthyroid patients as compared to normal controls, while the response to GHRP-6 was normal. In addition, there was absence of the normal synergistic effect of the two peptides in the hyperthyroid patients.

Critical illness

Protein hypercatabolism and preservation of fat depots are hallmarks of critical illness, which is associated with blunted pulsatile GH secretion (reduced pulse amplitude and elevated interpulse levels) and low circulating IGF-1, TSH, T4 and T3. Recently, repetitive TRH administration was found to reactivate the pituitary-thyroid axis and to evoke paradoxical GH release in severe illness (Van den Berghe *et al.*, 1996a).

Van den Berghe *et al* (1996b) studied the GH responses to GHRH, GHRP-2 and GHRH plus GHRP-2 in 40 severely ill patients, aged 14-81 years (median 62.5 years) who were admitted to the intensive care unit. The group was inevitably heterogenous so the study was designed to minimise the impact of confounding factors by investigating patients as their own controls over as brief as possible study period (total duration 8 hours). Following randomisation into four equal groups, patients received two i.v. boluses at six hourly intervals, as follows: placebo and GHRP-2, GHRH and GHRP-2, GHRP-2 and GHRH plus GHRP-2, or GHRH plus GHRP-2 and GHRH plus GHRP-2 plus TRH. There was a striking GH response to GHRP-2 (> 18-fold higher than after placebo), which was more than fourfold higher than the response to GHRH. In turn, the mean GH response to GHRH plus GHRP-2 was 2.5 higher than that to GHRP-2 alone, indicating synergism. Adding TRH to the GHRH plus GHRP-2 combination blunted this mean response by 18%. This study demonstrated that the specific character of hypothalamic-pituitary function in critical illness extended to responsiveness to GHRH and/or GHRP-2 and excluded a lack of pituitary GH releasing capacity as a mechanism underlying the blunted GH secretion during critical illness.

There is increasing evidence that recombinant exogenous GH administration in hypercatabolic states such as critical illness and severe sepsis attenuates protein catabolism (Ziegler *et al.*, 1990; Voerman *et al.*, 1992; Voerman *et al.*, 1995). In addition, GH has immunoregulatory

effects and may act to protect the host from lethal bacterial infections (Saito *et al.*, 1996). These properties, together with the beneficial effects of GH on wound healing and skeletal muscle function, may potentially accelerate recovery during critical illness. The findings of Van den Berghe *et al* (1996b) open perspectives for administration of GHRPs as a potential strategy to reverse the protein hypercatabolism in prolonged severe illness which contributes substantially to the morbidity and mortality of the condition. They also provide an alternative therapeutic approach to the administration of recombinant exogenous GH in such conditions.

Obesity

Obesity is associated with an impairment of GH secretion elicited by all stimuli known to date (Bell *et al.*, 1970; Glass *et al.*, 1981; Williams *et al.*, 1984; Dieguez *et al.*, 1988; Cordido *et al.*, 1990), but the basic mechanisms of this alteration are not yet clear. Although obese subjects exhibit an increase in GH clearance rate, it is widely accepted that the main alteration is a decrease in both spontaneous and stimulated pituitary GH secretion. The finding that the cholinergic agonist pyridostigmine, which putatively acts by inhibiting hypothalamic SS release, partially restored stimulated GH secretion in obese subjects (Cordido *et al.*, 1989) suggested the existence of increased SS tone in obesity.

Cordido *et al* (1993) studied the GH releasing effect of GHRP-6, GHRH and GHRP-6 plus GHRH in a group of obese patients weighing more than 130% of their ideal body weight. GHRP-6 induced a significantly greater GH response than GHRH, which lead, as expected, to only a slight increase in plasma GH levels. Pretreatment with pyridostigmine, given orally 60 minutes before GHRP-6, significantly increased the mean peak GH. The combined administration of GHRP-6 plus GHRH induced massive GH discharge which was synergistic. A correlation was evident between body mass index (BMI) and the GH secretion elicited by GHRP-6 alone ($r = -0.58$; $p = 0.03$) but not by GHRP-6 plus GHRH. There was correlation between BMI and GHRH-induced GH release. This work of Cordido *et al* (1993) showed GHRP-6 to be the most potent GH stimulus in obese patients. The synergistic GH response to the combined administration of GHRP-6 plus GHRH (> 40 μg/L) was described by the authors as "massive" because it was so much greater than had been previously observed (<

10 μg/L) in obese human subjects (Bowers, 1993b). These data ruled out a previous belief that the somatotroph cell was altered permanently in obesity and indicated that the GH blockade characteristic of obesity was a functional and potentially reversible state.

In a follow up study, Cordido *et al* (1995) examined the effect of pyridostigmine pretreatment on the GH response to the combined administration of GHRP-6 plus GHRH in obese and non-obese subjects. The combined administration of the two secretagogues resulted in a marked increase in plasma GH levels in both groups. However, the GH response in obese subjects was lower than in normal subjects. Pretreatment with pyridostigmine failed to alter the GH response in both groups. These data therefore suggested that the somatotroph cells in obesity have a considerable GH secretory capacity, although lower than in normal subjects. The reasons for the decrease in the GH-releasable pool present in obesity remains uncertain, but may be accounted for by an increase in free fatty acids or by other hormonal and metabolic alterations.

Loche *et al* (1995a) reported the effect of hexarelin and GHRH administration to a group of 10 obese children. All were prepubertal, of normal stature and had excess body weight of 47-86% above their ideal. The response to hexarelin was significantly greater than that to GHRH. However, compared to non-obese prepubertal children, the respective GH responses to GHRH and hexarelin were significantly lower in the obese group. These data are in keeping with the findings by Cordido *et al* (1993) discussed above.

The above account summarises the data available on the activity of GHRPs in disease states. It can be seen that, as well as their potent GH releasing activity in normal humans, GHRPs are active in a variety of pathological conditions in which GH production is altered.

Section 5. Diagnostic and Therapeutic Applications of Growth Hormone Releasing Peptides

Characteristics that have lead GHRPs to be considered for diagnostic and therapeutic uses include their potency, ease of administration, safety profile, minimal side-effects, activity in various age groups and various conditions (e.g. obesity) and the reproducibility of their actions. The activity of GHRPs following oral and intranasal administration is a clear advantage. This is particularly important in the therapeutic setting in children, where compliance with injectable recombinant human growth hormone (r-hGH) can be a problem (Smith *et al.*, 1993). For obvious reasons, GH deficiency states have been the main target for the diagnostic and therapeutic applications of GHRPs. Table 9 lists the diagnostic and therapeutic trials published so far. These are discussed in detail in this Section.

Diagnostic studies

The criteria for the diagnosis of GH insufficiency at different ages, based on provocative tests designed to test the integrity of the hypothalamopituitary axis, are controversial and lack a consensus (Rosenfeld *et al.*, 1995; Dattani *et al.*, 1992). Appropriate cut-off values need to be established for different assays and laboratories. The diagnosis in adults lacks a clear physiological marker of GH action at the tissue level, such as linear growth. The problem in the paediatric age group is compounded by the lack of normal data and the fact that tall, normal and short children have different GH secretion patterns (Albertsson-Wikland and Rosberg, 1988). Age and pubertal stage need also to be taken into account (Martha *et al.*, 1988).

Table 9. Diagnostic and therapeutic studies of GHRPs in children and adults.

a) Diagnostic studies

Age group	Type of GHRP	Route of administration	Reference
Children	hexarelin	i.v. and i.n.	Laron *et al.*, 1994
Children	GHRP-2	i.v. and i.n.	Pihoker *et al.*, 1995a
Children	GHRP-2	i.v.	Tiulpakov *et al.*, 1995b
Children	GHRP-1	i.v.	Laron *et al.*, 1993
Children	GHRP-1	i.v.	Mericq *et al.*, 1995a
Children	GHRP-6	i.v.	Pombo *et al.*, 1995a
Children	GHRP-6	i.v.	Pombo *et al.*, 1995b
Children and adults	hexarelin	i.v.	Loche *et al.*, 1995b
Adults	GHRP-6	i.v.	Leal-Cerro *et al.*, 1995
Adults	GHRP-6	i.v.	Popovic *et al.*, 1995

b) Therapeutic trials in children

Type of GHRP	Route of administration	Follow up period	Reference
Hexarelin	i.n.	8 months	Laron *et al.*, 1995a
GHRP-2	s.c.	6 months	Mericq *et al.*, 1995b
GHRP-2	i.n.	3-4 months	Pihoker *et al.*, 1995b

The above limitations make the comparison between different diagnostic studies of GHRPs difficult. An added problem with the studies reported so far is the small number of subjects recruited in each study. The lack of normal data for the different age groups necessitates the comparison of the GH responses to GHRPs with the responses to other provocative tests, preferably in age-matched normal controls, which introduces an ethical dimension to the problem. Indeed, most studies published so far have compared the effect of GHRPs with those of standard provocative tests, such as the insulin tolerance test (ITT), and pharmacological tests, such as the GHRH test. The latter is not as robust a test as other provocative tests because the GH responses to a single bolus dose of GHRH are extremely variable (Gelato *et al.*, 1984; Ghigo *et al.*, 1996b).

Since GHRPs are the most potent GH secretagogues and require intact GHRH to be fully active (see Section 3), it could be hypothesized that, at least in children, decreased GH response to GHRP in comparison to standard provocative tests may relate to the magnitude of endogenous GHRH deficiency. In such circumstances it would be important to determine whether the GH response to GHRP could be considerably greater when GHRP plus GHRH are administered together (Bowers *et al.*, 1992). Subjects with decreased GH responses to GHRP plus GHRH may require priming effects with one or both of these peptides to increase the GH response.

This approach, which may allow the identification of the most appropriate therapy for patients with GH deficiency, has been adopted by Bercu and Walker (1996). A pituitary function test was developed which compared responses to GHRH and GHRP administered sequentially and in combination. Robust GH secretion in response to GHRH or GHRP were interpreted as representing adequate endogenous GHRP and GHRH, respectively. Alternatively, a poor response to either GH secretagogue administered by itself represented inadequacy of its endogenous complement, assuming that all pituitary cellular and molecular elements for GHRH- and GHRP-mediated GH secretion were intact. The integrity of functional pituitary elements were differentiated from inadequate endogenous complement by administering both secretagogues together. The data resulting from the application of these principles would allow appropriate selection of therapeutic entities. Where a poor GH response to GHRH and

an adequate one to GHRP is observed, the latter would be chosen for therapy, and vice versa. If no response is observed following the administration of either secretagogue alone but an adequate one is seen following their concomitant administration, dual therapy (GHRH plus GHRP) would be used. If the latter failed to stimulate adequate GH release, indicating a lack of pituitary mechanism for GH production, exogenous recombinant GH therapy would be offered.

Although the studies referred to in this section may not have been conducted primarily for investigating the diagnostic potential of GHRPs, important inferences can be made from the data obtained.

a) Studies in children

Laron *et al* (1994) studied 10 children with familial short stature who had had a normal response to either clonidine or ITT (peak GH concentration > 20 mU/L). At a one week interval, each subject was given i.v. (1.0 μg/kg) or i.n. (20 μg/kg) hexarelin. Tests were performed in the morning in the fasted state. All subjects had a peak GH response greater than 20 mU/L. The peak response after i.v. administration occurred earlier (15-30 minutes) than after i.n. administration (30-60 minutes). Despite the small number of subjects in this study, it demonstrated that i.n. hexarelin was as effective a provocative test for pituitary GH secretion as i.v. hexarelin administration and compared well with other standard provocative tests.

Pihoker *et al* (1995a) compared the GH responses to GHRP-2 and GHRH with the responses to other provocative agents in 24 children with short stature. All children were prepubertal, had height standard deviation scores (SDS) < -2, had delayed bone age, height velocity (HV) < 25% for age, low serum IGF-1 concentrations and normal cranial MRI. Each child underwent at least one standard provocative test (arginine, ITT, L-dopa, or clonidine). Most children completed three standard tests. Normal response was defined as a peak GH concentration of greater than 10 μg/L. Children then underwent an i.v. GHRP-2 (1.0 μg/kg)

and GHRH (1.0 μg/kg) stimulation tests, one week apart, performed in the morning in the fasted state. A subgroup of 12 children were also given GHRH plus GHRP (1.0 μg/kg i.v. bolus of each peptide). Twenty one children had a peak GH response > 20 μg/L to both GHRH and GHRP-2. The remaining 3 children had received cranial irradiation for central nervous system (CNS) tumours or leukaemia and had a low response to GHRP-2 and a subnormal response to the standard provocative test (< 10 μg/L). Of the children who responded to GHRP-2, only 4 had a peak GH response greater than 10 μg/L in response to the standard provocative test(s). Of those who had a normal response to i.v. GHRP-2, a subgroup of 15 children were enrolled to undergo an i.n. GHRP-2 (dose range 5-20 μg/kg) stimulation test, to which all had a significant response (mean peak GH concentration 31.3 ± 6.0 μg/kg). Although this study contained a heterogenous group of short children, it allowed the exploration of a potential correlation of GH responses to standard agents with GH responses to GHRP-2. No such correlation could be found, and there were no differences in the response to GHRP-2 and GHRH. The combined administration of GHRP and GHRH was synergistic. It was concluded that i.v. and i.n. GHRP-2 were safe and effective in stimulating GH release in children with short stature, and that they were more sensitive than the conventional agents in assessing pituitary GH secretory ability or releasable GH pool.

In a study by Tiulpakov *et al* (1995b), comparison between standard provocative tests and i.v. GHRP-2 (1.0 μg/kg) was made in 9 short children with GH deficiency and 5 constitutionally short children [oral clonidine peak GH concentration < 5.4 μg/L, (range: 0.1-5.4 μg/L) and >7.3 μg/L [range: 7.3-34.9μg/L], respectively). All GH-deficient children but one had a low peak GH response to GHRP-2 (range: 0.1-1.5 μg/L, with one subject having a peak response of 30.1 μg/L). In contrast, the constitutionally short children had peak GH levels greater than those generated following standard provocative tests (range: 8.7-104 μg/L).

Laron *et al* (1993) studied a group of 8 adolescents, aged 18.3 ± 3.4 (mean ± SD) years, with abnormal GH secretion diagnosed following a provocative test. Six had GH deficiency of pituitary origin, 1 had partial GH deficiency (ITT and/or clonidine peak GH response < 3 μg/L and 5-8 μg/L, respectively) and 1 had hypothalamic GHRH deficiency, based on lack

of response to ITT and clonidine and a peak GH value of 10.5 μg/L to GHRH (1.0 μg/kg) stimulation. All patients were given i.v. GHRP-1 (1.0 μg/kg) in the morning following an overnight fast. The 6 patients with GH deficiency of pituitary origin and the patient with hypothalamic GH deficiency showed no response to GHRP-1 stimulation. The patient with partial GH deficiency had a peak GH response of 6.5 μg/L to GHRP-2, 7 μg/L to clonidine, 3.2 μg/L to ITT and 9.2 μg/L to GHRH (1.0 μg/kg). This study indicated that the GH response to GHRP-1 in GH deficiency states was dependent on the underlying aetiology of the disorder.

Mericq *et al* (1995a) compared the GH response to i.v. GHRP-1 (1.0 μg/kg), GHRH (1.0 μg/kg) and the two secretagogues administered together at the same above doses in 22 prepubertal children with idiopathic GH deficiency. The diagnosis of GH deficiency was based upon a peak GH response of less than 7 μg/L in two different GH stimulation tests (clonidine, L-dopa or ITT) and upon clinical evidence of growth failure. Each patient was tested after an overnight fast on three occasions separated by at least 1 week. A significant response to peptide administration was defined as a GH rise of greater than 4 assay SD. Table 10 summarises the results of the study:

Table 10. GH response to GHRP-1, GHRH and GHRP-1 plus GHRH in 22 prepubertal children with idiopathic GH deficiency.

GH secretagogue(s)	No. of responders	Peak response (μg/L)	Time to peak (minutes) (mean ± SD)
GHRP-1	12 (60%)	7.5 ± 8.0	25.4 ± 10.1
GHRH	15 (68%)	11.2 ± 12.1	30 ± 18
GHRH + GHRP-1	19 (86%)	34.2 ± 44.8	22.9 ± 13.6

There was no significant difference between the GH response to GHRH and GHRP-1. The magnitude of the response to the two secretagogues correlated poorly (r = 0.26). The combined administration of the two secretagogues resulted in a synergistic response. This study showed that the majority of children with GH deficiency can respond to bolus doses of

GHRP-1 with a brisk rise in GH, despite the absence of GH responses to most indirect stimuli. GH responses to GHRP-1 were, however, lower than those reported in normal adults, normal children and non-GH deficient children.

Pombo *et al* (1995a) compared the GH response to GHRP-6 (1.0 μg/kg) in 10 children with poor height velocity (HV) and GH deficiency. The latter was based on a peak GH concentration < 10 μg/L on provocative GH stimulation testing (propranolol and exercise, clonidine, or L-dopa). Peak GH responses to GHRP-6 ranged from 2.9-42.2 μg/L: 50% of the patients showed a GH response to GHRP-6 greater than 10 μg/L while the other 50% failed to do so. Peak GH concentration for the group was 13.6 ± 4.1 μg/L (mean ± SEM) compared to 25.3 ± 3.4 μg/L for a control group of 12 normal children. The authors concluded that i.v. GHRP-6 could be used as a test of pituitary GH reserve in short-statured children, but that, on an individual basis, it was not possible to distinguish between patients with idiopathic GH deficiency, as defined by standard provocative tests, and normal children. Limited conclusions could be drawn from this study since the provocative GH stimulation tests used were not the most robust available. Indeed, as discussed above, "standard" GH stimulation tests are known to be flawed.

Pombo *et al* (1995b) studied a group of 7 children and adolescents with GH deficiency secondary to neonatal pituitary stalk transection. The diagnosis was made on the basis of a history of perinatal abnormalities (breech delivery, neonatal hypoglycaemia, or low birth weight), short stature, abnormal growth rate, delayed bone age, absence of serum GH response to two stimulation tests (clonidine and L-dopa tests; peak serum GH concentration < 7 μg/L), low IGF-1 levels and MRI finding of transection of the pituitary stalk and the presence of a maldescended posterior lobe at the proximal stump. Pituitary dwarfism had been diagnosed at ages ranging from 4.0-9.9 years. Subjects were studied some years after the diagnosis had been made (age range 11.6-17.6 years) and were all receiving r-hGH replacement therapy, which was discontinued 7 days before they were reinvestigated. Each subject was given an i.v. bolus of GHRP-6 (1.0 μg/kg), GHRH (1.0 μg/kg), and GHRP plus GHRH (1.0 μg/kg of each) on three separate occasions, at least 4 days apart. Administration of GHRH did not stimulate GH release, with maximal levels ranging from 0.6-2.2 μg/L (peak

GH 1.8 ± 0.3 μg/L, mean ± SEM). Similarly, GHRP-6 was devoid of effects, with maximum GH increments ranging from 0.2-2.4 μg/L (peak GH 0.8 ± 0.2 μg/L). The GH stimulus after combined administration of GHRH plus GHRP-6 was severely blunted, with individual levels ranging from 0.8-4.7 μg/L (peak GH 3.0 ± 0.5 μg/L). The latter finding is interesting since it is the only situation studied so far in childhood where the combined administration of these two secretagogues failed to produce massive GH release, and thus may have important diagnostic implications. Although not discussed by the authors, results might have been different had the subjects been primed with GHRH or had r-hGH been stopped for a longer period before the peptides were administered, or indeed had the peripubertal subjects been primed with sex steroids before being studied.

Loche *et al* (1995b) studied the GH response to hexarelin in a group of 15 children with GH deficiency. All children had clinical features of GH deficiency and peak GH responses of < 10 μg/L after two GH stimulation tests (ITT, clonidine or arginine). The group was heterogenous for the aetiology of the GH deficiency (7 had idiopathic GH deficiency, 5 had pituitary stalk interruption syndrome, 2 had a pituitary cyst and 1 had empty sella syndrome). All patients had been receiving r-hGH therapy which was discontinued 2-4 weeks before the study. Five patients had associated pituitary hormone deficiencies and were on appropriate replacement therapy. Each subject was given i.v. hexarelin (2.0 μg/kg) and GHRH (1.0 μg/kg) separated by 3 to 7 days. All children with idiopathic GH deficiency responded to hexarelin, with a peak GH response range of 37-88 μg/L (63.0 ± 6.5 μg/L, mean ± SEM); the response was not significantly different from that observed in short normal children. In contrast, the GH response to GHRH ranged from 7.5-32.0 μg/L (15.1 ± 3.2 μg/L). The children with pituitary stalk interruption syndrome showed a poor GH response to hexarelin (peak response range 1.0-10.0 μg/L). The response of the latter group to GHRH stimulation was similar, except for one subject who attained a peak GH response of 25.0 μg/L. One child with pituitary cyst had a peak GH response of 26.0 μg/L to hexarelin stimulation while the other attained a peak of only 9.0 μg/L; their peak GH responses to GHRH stimulation were 38.0 μg/L and 15.0 μg/L, respectively. The child with empty sella syndrome showed no response to either secretagogue (peak response to hexarelin 0.3 μg/L; peak response to GHRH 0.9 μg/L). The authors concluded that hexarelin was capable of stimulating GH

secretion in patients with idiopathic GH deficiency and that it may, if their findings were confirmed in a larger number of patients, be useful for identifying the aetiology of the GH deficiency.

b) Studies in adults

In the above study by Loche *et al* (1995b) adults with GH deficiency were also studied. 1 had idiopathic GH deficiency while the remaining 3 had pituitary stalk interruption syndrome. All 4 had had GH deficiency confirmed on two provocative tests (ITT, clonidine or arginine, peak GH < 10 μg/L). The patient with idiopathic GH deficiency mounted a peak GH response of 88.0 μg/L to hexarelin stimulation and 6.2 μg/L to GHRH. The remaining 3 failed to show any response to either secretagogue. These findings were in keeping with those observed in the children reported in the same study and conclusions are hence similar (see above).

Leal-Cerro *et al* (1995) studied 21 adults with long-standing GH deficiency, aged 21-45 years. GH deficiency had been diagnosed on the basis of a peak GH response of < 10 mU/L following two provocative tests (ITT and L-dopa). Four patients had idiopathic GH deficiency, 1 had an empty sella turcica, and the remainder were studied following pituitary surgery for non-functioning pituitary adenoma (10 patients), Cushing disease (2 patients), Nelson syndrome (1 patient) or a craniopharyngioma (3 patients). Taking into the account that some subjects might have exhibited transient GH deficiency, all patients were submitted again to an ITT. None had a peak GH response of > 10 mU/L on retesting. All subjects underwent a combined i.v. GHRH (1.0 μg/kg) plus GHRP-6 (1.0 μg/kg) in the morning in the fasted state. Three out of the 4 patients with idiopathic GH deficiency had a marked peak GH response to the two secretagogues (100, 98 and 62 mU/L); the fourth had a blunted response of 1.2 mU/L. The remainder had a heterogenous pattern of response. Five had a response ranging from 11.0-14.6 mU/L while the remaining 12 had a markedly reduced response of less than 10 mU/L. This study demonstrated that GHRP-6 combined with GHRH was capable of eliciting a normal GH response in 40% of adults diagnosed with GH deficiency by conventional GH testing. However, the GH response to GHRP seems more likely in cases of idiopathic GH deficiency.

Popovic *et al* (1995) studied a group of 12 adults with different neuroendocrine pathologies leading to a state of hypothalamopituitary disconnection (functional stalk section). The diagnosis of hypothalamopituitary disconnection was made on the basis of clinical features and evidence of lesion in the hypothalamopituitary area by MRI, plus different degrees of hypopituitarism with normal or delayed pituitary response after the administration of TRH, GnRH and GHRH. Six patients showed, in addition, moderate hyperprolactinaemia. The authors discussed in their manuscript the controversies, problems and difficulties encountered in diagnosing hypothalamopituitary disconnection. All patients had received GH replacement therapy many years before they were restudied. Each patient underwent stimulation tests with i.v. GHRH (100 μg), GHRP-6 (90 μg) and GHRH (100 μg) plus GHRP-6 (90 μg). The GH response to GHRH (5.6 ± 0.8, mean ± SEM) was similar to that obtained in a group of sex- and age-matched controls (8.8 ± 1.9 μg/L) but showed a delayed secretory curve. The peak GH response to GHRP-6 was markedly reduced (1.6 ± 0.1 μg/L *vs* 28.3 ± 6.0 μg/L in controls). Individually studied, each of the 12 patients presented a GH response that was 50% or less than the secretion elicited by GHRH. The combined administration of the two secretagogues resulted in an impaired peak GH response which was merely additive (11.6 ± 1.2 μg/L) compared with the synergistic response observed in normal controls (59.3 ± 8.1 μg/L). The authors suggested that 50% less GH release after GHRP-6 than after GHRH could be used as test of hypothalamopituitary disconnection in adults, though further larger sample size studies were needed.

The studies of GHRPs in acromegalic subjects (see Section 4) showed that the GH response to GHRP was qualitatively similar to that in healthy adults. Thus, GHRPs are unlikely to be useful diagnostically in acromegaly.

It is clear from the above studies that GHRPs may well have a role as safe, well-tolerated and reliable tests of pituitary GH reserve. Further studies, with larger sample size and more uniform pathological entities, are needed to determine the sensitivity and specificity of the tests. Large field studies are also needed to get the natural variability and to avoid bias associated with selecting disease only cases. Defining the appropriate dose of GHRP for the route by which it is to be administered, as well as defining the appropriate sampling interval

and duration of sampling are important issues which need to be standardised. These should be relatively straight forward considering that data on bioavailability and pharmacokinetics are known (see Section 2), though dose-response studies are lacking. Given our lack of understanding of the exact site and mechanism of action of GHRPs, such tests cannot establish the precise aetiology for a GH deficiency state. In practical terms this may not be essential for instituting therapy. A GH response to GHRPs and a lack of response to other GH stimulation tests in the clinical setting of suspected GH deficiency may be an indication for therapy with GHRPs.

Therapeutic studies

As already discussed in previous sections, GHRPs are undoubtedly potent GH secretagogues. Their ability to induce GH release following acute bolus and continuous infusions has already been discussed. Short term studies have shown some evidence that the GH response to GHRPs may down regulate with chronic administration (DeBell *et al.*, 1991; Huhn *et al.*, 1993; Laron *et al.*, 1995b), a potential draw back if they were to be used in long-term therapy. What remains to be seen is whether GHRPs prove to be efficacious as therapeutic agents. To date, only a limited number of studies of the therapeutic application of GHRPs have been conducted (see Table 9).

a) Studies in Children

Children with short stature and/or growth hormone deficiency were an obvious target for therapeutic trial of GHRPs. The three studies conducted so far involve small numbers of children making statistical analysis difficult. Moreover, the follow up period remains short and conclusions about the longer term therapeutic responses and potential side-effects cannot be drawn at this stage. The inclusion criteria of the subjects and the type, dose and mode of administration of GHRPs used are different in the three studies, thus making comparison between them difficult.

Laron *et al* (1995a) studied a group of 8 prepubertal, constitutionally-short children with

normal GH responses to clonidine and/or ITT and a GH response of 40-60 mU/L to one i.n. dose (20 μg/kg) of hexarelin. All children were treated with i.n. hexarelin (60 μg/kg t.d.s.). Follow up period was short, ranging from 3 to 8 months. Height velocity (HV) increased in all children; for the entire group this was 8.3 ± 1.7 cm (mean ± SD) compared with a pretreatment HV of 5.4 ± 0.8 cm ($p < 0.0001$). Additional findings included a significant rise in serum IGF-1, alkaline phosphatase and inorganic phosphate concentrations. Bone age, assessed at 6 months, had advanced less than or in parallel with chronological age. Intranasal hexarelin was very well tolerated and caused no local irritation. No undesirable effects were observed or reported.

Mericq *et al* (1995b) investigated the therapeutic effect of GHRP-2 in 6 prepubertal GH-deficient children. Growth hormone deficiency was defined as HV below 4 cm/year, significantly delayed bone age and maximum GH response to two provocative tests of less than 4 μg/L. Children were treated with s.c. GHRP-2 at increasing doses every two months: 0-2 months 0.3 μg/kg/day, 2-4 months 1.0 μg/kg/day and 4-6 months 3.0 μg/kg/day. Follow-up period was 6 months. Children were assessed at 2 monthly intervals. By the end of the 6 month treatment period the HV had increased from 2.5 ± 0.5 cm/year (mean ± SD) to 5.6 ± 1.5 cm/year ($p < 0.05$). Mean peak GH concentration, obtained from measured serum GH during a 20-minute interval overnight profile, had increased from 12.7 ± 11 μg/L to 34.6 ± 28 μg/L ($p < 0.05$). There were no clinical or biochemical adverse reactions during the course of the study.

Pihoker *et al* (1995b) treated a group of 15 GH-deficient children with i.n. GHRP-2. Doses of 5-15 μg/kg, initially twice then three times daily, were used. Follow up period was 3-4 months. Height velocity increased from 3.6 ± 0.2 cm/year (mean ± SEM) to 6.7 ± 0.8 cm/year. There was no significant change in serum IGF-1 or IGF-BP3 concentrations. Acute GH responses to both i.v. and i.n. bolus GHRP-2 tended to be higher than pretreatment, although the changes were not statistically significant. No adverse findings were noted on direct inspection of the nasal mucosa.

Although the above studies provide encouraging preliminary results, it is noteworthy that the

improvement in height velocity reported in the studies by Mericq *et al* (1995b) and Pihoker *et al* (1995b) is of a lesser magnitude than that expected in children with true GH deficiency treated with exogenous GH (Prader *et al.*, 1967).

b) Studies in adults

No formal therapeutic studies of GHRPs have been reported to date. Indeed, the use of GH therapy in adult life is a relatively recent development. In the United Kingdom, r-hGH was licensed for use in adult GH deficiency syndrome only in 1996. The long term effects and benefits of GH replacement in adults are still unknown, though short-to-medium term effects have been reported (Burman *et al.*, 1995; Hansen *et al.*, 1995; Christiansen *et al.*, 1996). By means of their potent GH releasing activity in normal young and elderly subjects and in adults with growth hormone deficiency, GHRPs may prove to be important therapeutic agents outside the childhood age range.

The potent GH releasing activity of GHRPs, alone or in combination with GHRH, in various disease states discussed in Section 4 of this thesis may be utilised in future therapeutic trials to reverse the adverse effect of GH insufficiency. An interesting area would be the use of GHRPs in catabolic states, such as critical illness, where GHRPs (alone and in combination with GHRH) have been shown to induce massive GH release despite the presence of a number of normally inhibiting factors, namely, a non-fasting state and elevated serum cortisol and blood glucose levels (Van den Berghe *et al.*, 1996b).

Summary

GHRPs are methionine-enkephalin analogues developed by Bowers and co-workers in the late 1970s. They consist of a small number (<7) of amino acids and are potent GH secretagogues in many animal species, including man. So far, four different GHRPs have been developed and administered to man. Non-peptidyl GH secretagogues have been modeled on GHRPs and newer and smaller GHRPs are being developed. An interesting and important feature of GHRPs is their activity following various routes of administration. Although initially thought to be specific for GH release, GHRPs have been shown to induce PRL and cortisol release both *in vitro* and *in vivo*.

Many studies have targeted the understanding of the site and mechanism of action of GHRPs. No consensus has been reached on the relative importance of the pituitary and hypothalamic action of GHRPs. Particular emphasis has been placed on the interaction and the interdependence of GHRPs and GHRH, while the interaction with SS has been much less extensively studied. The GHRP receptor has been recently cloned and its distribution studied. The intracellular action of GHRPs involves stimulation of phosphatidylinositol turnover which leads to activation of protein kinase and increase in intracellular Ca^{2+}. GHRPs seem to act primarily as releasers of GH rather than promoters of its synthesis.

The potent GH-releasing property of GHRPs, their safety and, most importantly, their activity following oral administration have made them an obvious choice for future therapeutic use. Indeed, therapeutic trials are already in progress at the time of writing. The preliminary data from these studies are promising, but long-term data are lacking.

Despite the numerous studies of GHRPs in the last ten years, a number of important issues relating to the potential physiological role and therapeutic applications of GHRPs have not been addressed. This thesis addresses some of these issues which include the effect of repeated administration of GHRP, alone or in combination with GHRH, the effect of GH feedback on the GH response to GHRP, the effect of varying SS tone and the effect of its

withdrawal on the GH response to GHRP, and the effect of varying doses of GHRP on the GH, PRL and cortisol response. The data obtained from these studies would help our understanding of the potential physiological role of GHRPs in the control of GH secretion. In addition, these data would form the basis for taking the GHRP hexarelin to the next stage of studies, namely, therapeutic trials.

CHAPTER 2

METHODS

Section 1. Subjects

The subjects recruited for all the studies were normal adult male volunteers. They were recruited by means of poster advertisement at the Middlesex Hospital. All were healthy, had no relevant past medical history, had no symptoms or signs of endocrine disorders and were not on any medication during the study period. None had recently participated in any other clinical trial. Their ages ranged from 20 - 35 years. Although some were smokers and had moderate alcohol consumption, they were not allowed to smoke or drink alcohol during any of the studies. They were all of normal stature and weight. Their body mass index (BMI) ranged from 19.6 - 26 kg/m^2, although the BMI of one subject who was studied over a period of two years increased to 28.9 kg/m^2 by the end of the study period. This was attributed to dietary overindulgence and the subject remained well otherwise. Subjects did not report any unusual symptomatology during the course of the study.

Female subjects were excluded in order to avoid any influences of fluctuating oestrogen levels on endogenous GH secretion or on the GH response to GHRP. Elderly or obese subjects were excluded because of their possible altered GH secretion pattern. Children were not included for ethical reasons. Subjects with a past medical history of endocrine disorders, pituitary surgery, significant head trauma, cranial irradiation, epilepsy or drug hypersensitivity were excluded.

Participation was purely voluntary and subjects were made aware of their right to withdraw from the studies at any stage. Four sets of studies were performed (see next Section - "Studies"). Some subjects participated in more than one set, with a gap of a few weeks between each. The list of subjects and the studies which they undertook are shown in Table 11.

Table 11. List of subjects and the studies which they undertook.

Subjects' initials	Studies undertaken
SD	Sets 1 and 2
PL	Sets 1, 2 and 3a
RT	Sets 1, 2 and 3b
MH	Sets 1, 3a, 3b and 4
RR	Sets 1, 3a, 3b and 4
RH	Sets 1, 3a and 4
JT	Set 2
AJ	Set 2
PJ	Sets 2 and 3b
KW	Sets 3a and 3b
AD	Sets 3a and 4
DS	Sets 3b and 4
JA	Set 4

Set 1. The effect of single and repeated doses of hexarelin, GHRH and hexarelin plus GHRH on GH secretion.

Set 2. The effect of GH feedback on hexarelin-induced GH release.

Set 3. The effect of somatostatin tone (set 3a) and somatostatin withdrawal (set 3b) on the GH response to hexarelin, GHRH and hexarelin plus GHRH.

Set 4. Dose-response studies of the GH-, PRL- and cortisol- response to hexarelin.

Section 2. Studies

All studies were approved by the Research Ethics Committee of the Middlesex Hospital (approval number 89/72). Subjects were given an information sheet detailing the purpose and nature of the studies. This was supplemented with a discussion relating to the set of studies volunteered for, following which written consent was obtained. Where a subject took part in more than one set of studies, the design of each set was explained and a separate written consent obtained.

The studies are divided into four main groups designed to answer several important issues relating to the physiology of the GHRP hexarelin. Hexarelin had been developed recently by Deghenghi *et al* (1992) and preliminary animal studies had shown it to be more potent than GHRP-6 (Deghenghi *et al.*, 1994). Hexarelin was found to be more lipophilic than GHRP-6, thus enhancing its rate of absorption. It was also found to be more resistant to metabolic degradation (Deghenghi *et al.*, 1994). It was therefore chosen for physiological studies in man in order to take it to the next stage of investigation, namely, therapeutic studies.

The following sets of studies were performed:

1) The effect of single and repeated doses of hexarelin, GHRH and hexarelin plus GHRH on GH secretion (set 1).

2) The effect of GH feedback on hexarelin-induced GH release (set 2).

3) The effect of somatostatin tone and somatostatin withdrawal on the GH response to hexarelin, GHRH and hexarelin plus GHRH (sets 3a and 3b)

4) Dose-response studies of the GH-, PRL- and cortisol- response to hexarelin (set 4).

1) The effect of single and repeated doses of hexarelin, growth hormone releasing hormone and hexarelin plus growth hormone releasing hormone on growth hormone secretion

The aim of this set of studies (set 1) was to determine the GH-releasing activity of a single bolus of intravenous hexarelin and to compare it to that of GHRH. The GH responses to two intravenous boluses of hexarelin separated in time by two different intervals (60 and 120 minutes) were investigated and compared to the those of GHRH. The GH responses to the coadministration of hexarelin plus GHRH under the same experimental conditions were also determined.

Subjects

6 healthy adult males, aged 25.4 - 34.1 years with BMI 21.4 - 26.0 kg/m^2, were studied.

Study design

Subjects were fasted from midnight. At 0830 h (t = -60 minutes), an indwelling intravenous cannula was inserted in each forearm; one cannula was used for blood sampling and the other for drug administration. Blood specimens for measurement of serum GH concentrations were collected at 15 minute intervals from 0900 h (t = -30 minutes) to 1230 h (t = 180 minutes). Intravenous boluses of 0.9% saline, hexarelin, GHRH-(1-29)-NH_2 or hexarelin plus GHRH-(1-29)-NH_2 were administered at 0930 h (t = 0 minutes) and at 1030 h (t = 60 minutes) or 1130 h (t = 120 minutes), according to the study design shown in Figure 6. Dosages of 1.0 $\mu g/kg$ of hexarelin and GHRH-(1-29)-NH_2 were used throughout. Each subject underwent 10 studies, outlined in Figure 6, performed in a random order with a washout period of at least two days between each.

Statistics

The mean and SEM of the peak serum GH concentrations following the first bolus of saline or secretagogue(s) were calculated and used to illustrate and compare the magnitude of the response to a single intravenous bolus. Comparison between non-paired data was made using the Mann-Whitney test and between paired data using the Wilcoxon matched pairs signed rank sum test.

The data were largely analysed in terms of GH secretion rates which were calculated from the measured serum GH concentrations by deconvolution analysis using a variable disappearance time estimate for GH (Hindmarsh *et al.*, 1990).

Two-way analysis of variance (two-way ANOVA) on a repeated measures design, using the subjects as a blocking variable and Scheffe's procedure to correct for multiple comparisons, was used:

a) to compare the peak GH secretion rates following the administration of the first intravenous bolus of secretagogue(s) (at t = 0 minutes)

b) to compare the peak GH secretion rates following the administration of the second intravenous bolus of secretagogue(s) (at t = 60 minutes *or* t = 120 minutes) with the corresponding response to intravenous saline.

The Wilcoxon matched pairs signed rank sum test (two-tailed) was used:

a) to compare the additive effect of the two secretagogues (the arithmetic sum of the respective peak GH secretion rates following their isolated administration) with their synergistic effect (the effect of their simultaneous administration).

b) to compare the peak GH secretion rates following the administration of the second intravenous bolus of secretagogue(s) (at t = 60 minutes *or* t = 120 minutes) with those

following the same secretagogue(s) administered at t = 0 minutes on the same study day. For the purpose of this set of statistical analyses the GH peak secretion rates following the second bolus (X) were corrected for residual secretory effect of the first bolus. This was achieved by determining the point in time at which X occurred, determining the GH secretion rate at the same point in time in the corresponding study in which saline was administered in place of secretagogue (Y) and then subtracting Y from X to obtain the corrected GH peak secretion rate. This process was particularly pertinent in the studies in which hexarelin was administered because of the persistence of its GH secretory activity beyond 60 minutes, and was made possible by the low intra-individual variability of the GH response to hexarelin (see below).

c) to test the hypothesis that the two secretagogues lost their synergistic effect when administered repeatedly. This was performed by comparing the arithmetic sums of the corrected GH secretion rates following the second bolus of each secretagogue at t = 60 minutes and t = 120 minutes with the corresponding corrected peak GH secretion rates following their simultaneous administration.

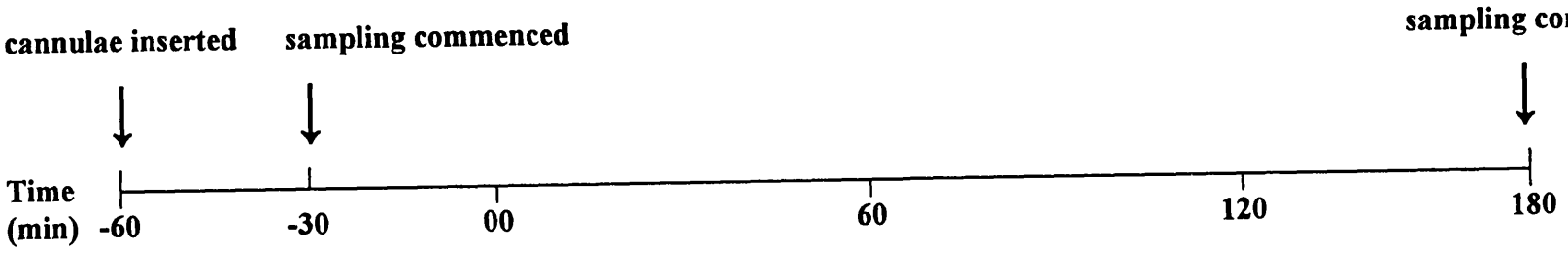
One-way ANOVA was used to compare the time to peak serum GH concentration following the bolus administration of hexarelin, GHRH-(1-29)-NH_2 or hexarelin plus GHRH-(1-29)-NH_2.

The mean and standard deviation (SD) of the intra- and inter-individual variability in the peak GH response to i.v. hexarelin (1.0 μg/kg) and GHRH-(1-29)-NH_2 (1.0 μg/kg) were calculated using the data from all four sets of studies described in this Section ("Studies"), as follows:

a) Intra-individual variability. Assessment of the intra-individual variability was possible because a number of subjects had undergone two or more complete sets of studies which contained sessions in which only hexarelin or GHRH-(1-29)-NH_2 (1.0 μg/kg of each) was administered intravenously. The coefficient of variance (CV), defined as the ratio of the SD to the mean of the peak GH response observed in any one individual following the i.v. administration of 1.0 μg/kg hexarelin or GHRH-(1-

29)-NH_2 (CV = SD ÷ mean), was calculated for each individual. The mean and SD of the CVs thus derived were then calculated to give the intra-individual variability of the peak GH response to hexarelin and GHRH-(1-29)-NH_2.

b) Inter-individual variability. Assessment of the inter-individual variability was possible because the different sets of studies contained sessions in which only hexarelin or GHRH-(1-29)-NH_2 (1.0 μg/kg of each) was administered intravenously, and all six subjects underwent these sessions. The coefficient of variance (CV), defined as the ratio of the SD to the mean of the peak GH response observed in the six subjects for each study session in which only i.v. hexarelin (1.0 μg/kg) or GHRH-(1-29)-NH_2 (1.0 μg/kg) was administered (CV = SD ÷ mean), was calculated for each study session. The mean and SD of the CVs thus derived were then calculated to give the inter-individual variability of the peak GH response to hexarelin and GHRH-(1-29)-NH_2.



Study number			
1	saline	saline	saline
2	hexarelin	saline	saline
3	hexarelin	hexarelin	saline
4	hexarelin	saline	hexarelin
5	GHRH	saline	saline
6	GHRH	GHRH	saline
7	GHRH	saline	GHRH
8	hexarelin + GHRH	saline	saline
9	hexarelin + GHRH	hexarelin + GHRH	saline
10	hexarelin + GHRH	saline	hexarelin + GHRH

Figure 6. Study design for set 1. An i.v. bolus of saline, hexarelin, GHRH-(1-29)-NH_2, or hexarelin + GHRH-(1-29)-NH_2 was administered at t = 0 min (studies 1, 2, 5 & 8, respectively). The studies were repeated on two further occasions, with a second identical bolus to that at t = 0 min administered at t = 60 min (studies 3, 6 & 9, respectively) or at t = 120 min (studies 4, 7 & 10, respectively). Secretagogue doses of 1.0 μg/kg were used throughout.

2) The effect of growth hormone feedback on hexarelin-induced growth hormone release

The aim of this set of studies (set 2) was to investigate the effect of GH feedback on the GH-releasing activity of hexarelin and to compare it to the effect on GHRH-induced GH release.

Subjects

6 healthy adult males, aged 23.8 - 34.3 years with BMI 19.6 - 25.5 kg/m^2, were studied.

Study design

Following an overnight fast, two indwelling intravenous cannulae were inserted in the forearms at 0830 h (t = -60 minutes), one for drug administration and the other for blood sampling. Intravenous boluses of 0.9% saline, hexarelin (1.0 μg/kg) or GHRH-(1-29)-NH_2 (1.0 μg/kg) were administered at t = 90 minutes, having administered an intravenous bolus of either 0.9% saline or r-hGH (100 mU) at t = 0 minutes, as shown in Figure 7. Blood specimens for the measurement of serum GH concentration were collected immediately following cannulation (t = -60 minutes) and then at 15 minute intervals for a total of four hours (t = -60 minutes to t = 180 minutes). Additional samples were drawn at t = 0 and t = 90 minutes for the measurement of serum IGF-1 levels.

In order to determine the peak serum GH concentration achieved following the bolus injection of r-hGH at t = 0 minutes, the sampling frequency was increased to every 5 minutes for 15 minutes immediately following this injection.

Blood specimens obtained from this group of studies were also used in the preliminary investigation of the effect of hexarelin on other anterior pituitary hormones. Using blood specimens collected in the studies where no r-hGH was administered (studies 1-3), serum

PRL, cortisol and TSH concentrations were measured at 15 minute intervals starting 30 minutes before the intravenous administration of 0.9% saline, hexarelin or GHRH-(1-29)-NH_2 (t = 90 minutes) and for 90 minutes thereafter (t = 180 minutes).

Each subject underwent six studies performed in a random order with a washout period of at least two days between each.

Statistics

The Mann-Whitney test was used to compare the peak serum GH concentrations following hexarelin administration with those following GHRH-(1-29)-NH_2, for the same previous bolus given at t = 0 minutes (saline or r-hGH).

The Wilcoxon matched pairs signed rank sum test (two-tailed) was used to compare two groups of paired data, namely, the effect of prior administration of r-hGH, as opposed to placebo, on the peak serum GH response to hexarelin and GHRH-(1-29)-NH_2.

One way analysis of variance (one-way ANOVA) was used:

a) to compare the serum IGF-1 concentrations just before and 90 minutes after the bolus injections of saline and r-hGH

b) to compare the peak GH concentrations in the circulation *immediately* following the bolus administration of r-hGH at time t = 0 minutes. These peak GH values were calculated by plotting the natural log transformed measured serum GH concentrations obtained at 5 minute intervals for the first 15 minute period against the time of sample collection. The relationship between time and the transformed data was described by linear regression. The serum GH concentration at the point t = 0 minutes was represented by the anti-$\log_e$ of the intercept of the regression.

Serum prolactin, cortisol and Thyroid Stimulating Hormone data

Serum PRL, cortisol and TSH concentrations at t = 90 minutes (just before intravenous administration of saline, hexarelin or GHRH-(1-29)-NH_2) were taken as baseline values and were compared with the maximum change (peak/trough) in their respective concentrations following the intravenous bolus at t = 90 minutes. Comparisons were made using the Wilcoxon matched pairs signed rank sum test (two-tailed).

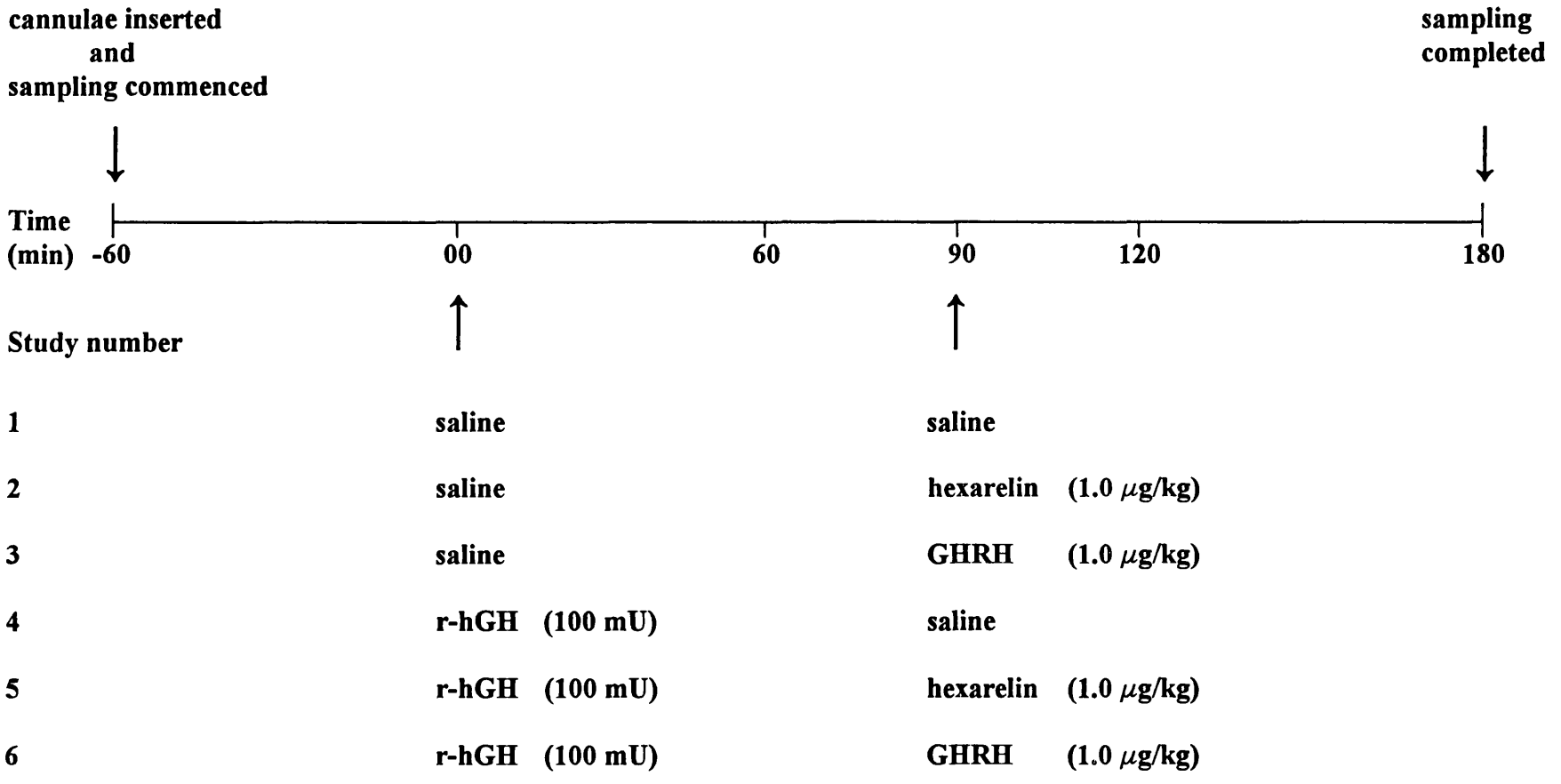


Figure 7. Study design for set 2. An i.v. bolus of either saline (studies 1, 2 & 3) or r-hGH (100 mU) (studies 4, 5 & 6) was administered at t = 0 min, followed by a second i.v. bolus of saline, hexarelin (1.0 μg/kg) or GHRH-(1-29)-NH_2 (1.0 μg/kg) at t = 90 min.

3) The effects of somatostatin tone and somatostatin withdrawal on the growth hormone response to hexarelin, growth hormone releasing hormone and hexarelin plus growth hormone releasing hormone

The aim of this set of studies (set 3) was to investigate whether increasing SS dose or SS withdrawal had any effect on the GH response to hexarelin, GHRH or hexarelin plus GHRH. A subset of the SS withdrawal studies was designed to investigate indirectly the ability of hexarelin to influence GH synthesis, as explained below.

Subjects

Two groups of 6 healthy adult males, aged 20.3-34.6 years with BMI 20.1-27.1 kg/m^2 were studied.

Study design

Following an overnight fast, two indwelling intravenous cannulae were inserted in the forearms at 0830 h (t = -60 minutes). One cannula was used for slow intravenous infusion of 0.9% saline or SS, which was commenced at t = -60 minutes, and the other for bolus administration of saline or drug(s) and for the collection of blood specimens. Two groups of studies were performed:

a) Studies of somatostatin dose

The 6 subjects in this group of studies (set 3a) were aged 28.7-34.6 years and had BMI 21.4-27.1 kg/m^2. 0.9% saline, SS(1-14) 20 $\mu g/m^2/hr$ (SS20) or SS(1-14) 50 $\mu g/m^2/hr$ (SS50) was infused from t = -60 minutes to t = 120 minutes. At t = 0 minutes, a bolus of 0.9% saline,

hexarelin (1.0 μg/kg), GHRH-(1-29)-NH_2 (1.0 μg/kg) or hexarelin (1.0 μg/kg) plus GHRH-(1-29)-NH_2 (1.0 μg/kg) was administered intravenously (Figure 8). Blood specimens for measurement of serum GH concentration were collected at 15 minutes intervals from t = -30 minutes to t = 120 minutes.

b) Studies of somatostatin withdrawal

The 6 subjects in this group of studies (set 3bi) were aged 20.3-34.3 years and had BMI 20.1-25.4 kg/m^2. 0.9% saline or SS20 was infused intravenously from t = -60 minutes to t = 120 minutes. At t = 120 minutes, the infusion was discontinued and a bolus of 0.9% saline, hexarelin (1.0 μg/kg), GHRH-(1-29)-NH_2 (1.0 μg/kg) or hexarelin (1.0 μg/kg) plus GHRH-(1-29)-NH_2 (1.0 μg/kg) was administered intravenously (Figure 9a). Blood specimens for measurement of serum GH concentration were collected at 15 minutes intervals from t = -30 minutes to t = 120 minutes, and at 5 minute intervals from t = 120 minutes to t = 180 minutes.

Four of these subjects underwent a further set of studies (set 3bii), the aim of which was to determine the ability of hexarelin to influence GH synthesis which might be reflected in the GH response to SS withdrawal. This was based on studies showing that GHRH stimulates GH synthesis during periods of high SS tone and allows the release of stored GH pools when SS tone is reduced (Kraicer *et al.*, 1986; Miki *et al.*, 1988; Clark *et al.*, 1988). In this set of studies, SS(1-14) was infused intravenously at a higher dosage of 50 μg/m^2/hr (SS50) from t = -60 minutes to t = 120 minutes. At t = 0 minutes a bolus of 0.9% saline, hexarelin (0.5 μg/kg) or GHRH-(1-29)-NH_2 (0.5 μg/kg) was administered intravenously (Figure 9b). Blood specimens for measurement of serum GH concentration were collected at 15 minute intervals from t = -30 minutes to t = 120 minutes, and at 5 minute intervals from t = 120 minutes to t = 180 minutes. The rationale for the higher dose of SS(1-14) was to ensure adequate suppression of GH secretion. Half-maximal doses of hexarelin (Imbimbo *et al.*, 1994) and GHRH-(1-29)-NH_2 (Spoudeas *et al.*, 1994) were used to avoid potential depletion of pituitary GH reserves.

All the above studies were conducted in a random order with a washout period of at least 72

hours between each. No side-effects were observed following the injection of hexarelin or infusion of SS. All subjects experienced transient facial flushing following the administration of GHRH-(1-29)-NH_2.

Statistics

a) Studies of somatostatin dose

The mean and SEM of the serum GH concentrations at each sampling point before and following the intravenous bolus administration of hexarelin during the constant intravenous infusion of saline, SS20 or SS50 (Figure 8, studies 2, 6 and 10) were calculated and plotted against the time of sample collection.

The mean and SEM of peak serum GH concentrations following bolus intravenous administration of saline or secretagogue(s) at t = 0 minute were calculated and used for presentation. The maximum serum GH concentration measured during the period t = -30 minutes to t = 0 minutes was determined and mean and SEM calculated. Statistical comparison was performed with one-way ANOVA. The Wilcoxon matched pairs signed rank sum test was used to compare serum GH concentrations between groups of paired data.

b) Studies of somatostatin withdrawal

Set 3bi

The mean and SEM of the serum GH concentrations at each sampling point during and following the withdrawal of the constant intravenous infusion of saline or SS20 and the intravenous bolus administration of hexarelin at the time of infusion withdrawal (Figure 9a, studies 2 and 6) were calculated and plotted against the time of sample collection.

The mean and SEM of peak serum GH concentrations and the time taken to reach this peak following cessation of saline or SS(1-14) infusion and the bolus intravenous administration

of saline or secretagogue(s) at t = 120 minutes were calculated and used for presentation. Statistical comparison between paired groups was performed using the Wilcoxon matched pairs signed rank sum test.

Set 3bii

Analysis of the data for these studies (shown in Figure 9b) was by performed by calculating and comparing the mean and SEM of the peak serum GH concentrations following the bolus administration of hexarelin or GHRH at t = 0 minutes, and those following the withdrawal of the SS50 infusion at t = 120 minutes.

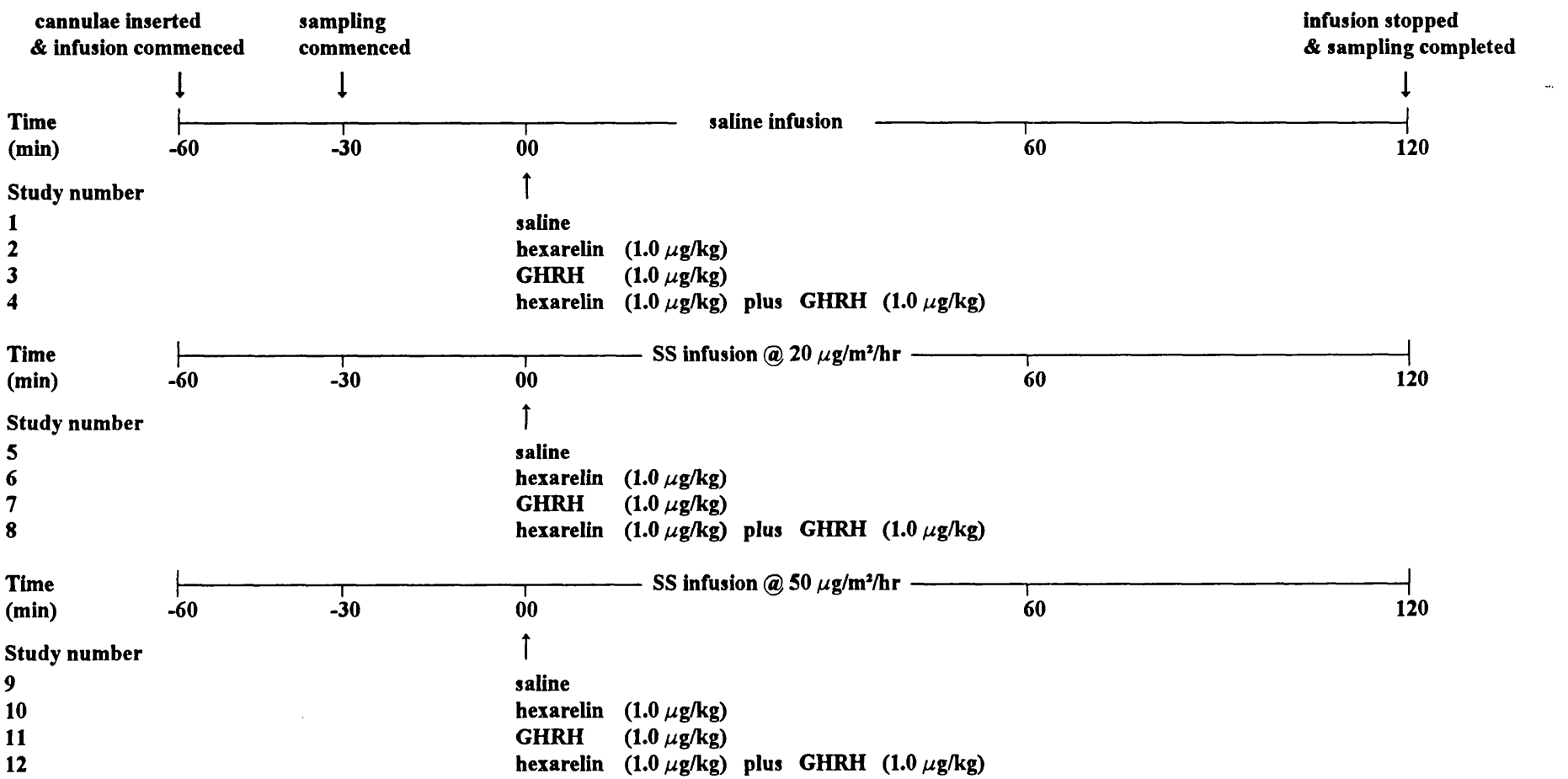


Figure 8. Study design for set 3a. Saline, SS (20 μg/m^2/hr) or SS (50 μg/m^2/hr) was infused intravenously from t = -60 to t = 120 min. During each infusion an i.v. bolus of saline, hexarelin (1.0 μg/kg), GHRH-(1-29)-NH_2 (1.0 μg/kg), or hexarelin (1.0 μg/kg) plus GHRH-(1-29)-NH_2 (1.0 μg/kg) was administered at t = 0 min. Blood specimens were collected at 15 minute intervals from t = -30 to t = 120 min.

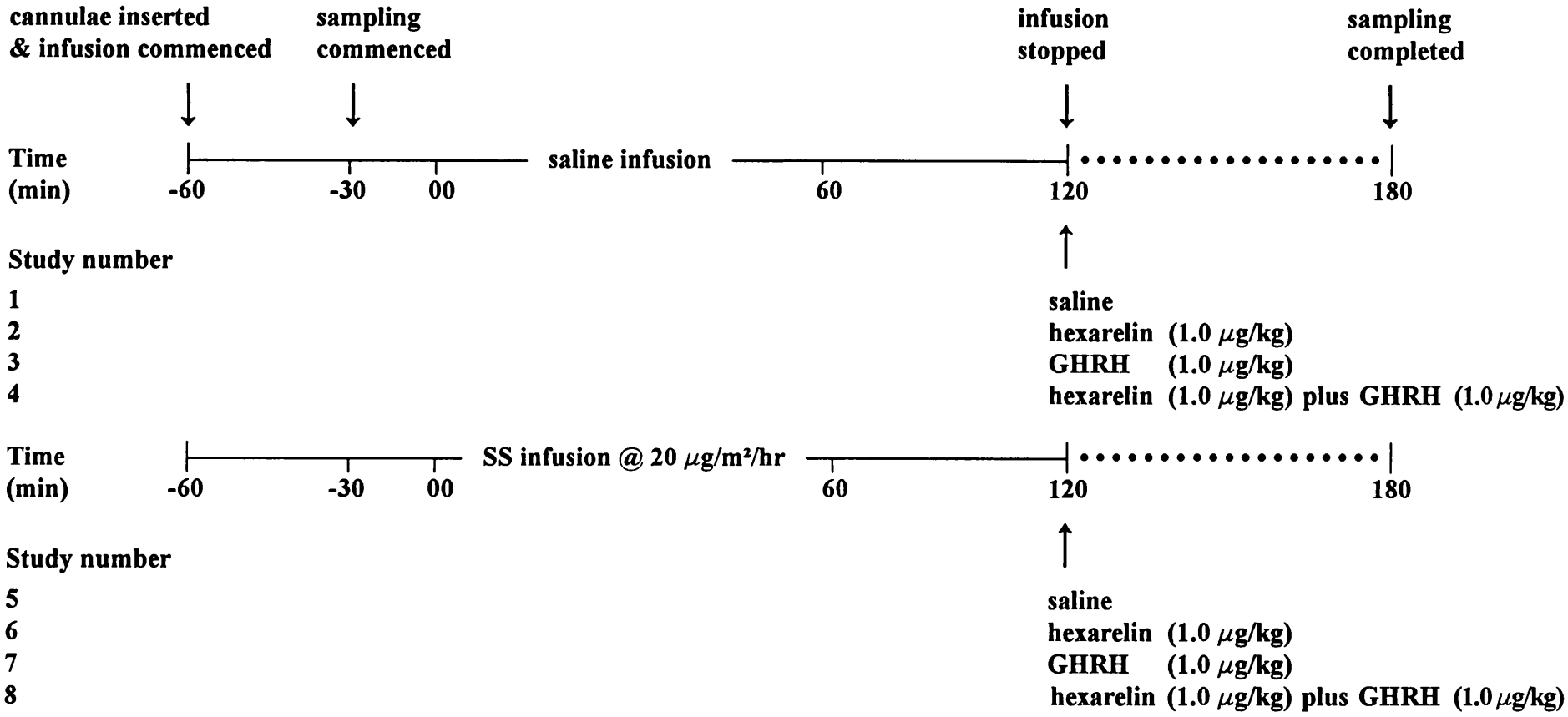


Figure 9a. Study design for set 3bi. Saline or SS (20 $\mu g/m^2/hr$) was infused intravenously from t = -60 to t = 120 min. At t = 120 min, the infusion was stopped and an i.v. bolus of saline, hexarelin (1.0 μg/kg), GHRH-(1-29)-NH_2 (1.0 μg/kg), or hexarelin (1.0 μg/kg) plus GHRH-(1-29)-NH_2 (1.0 μg/kg) was administered. Blood specimens were collected at 15 minute intervals from t = -30 to t = 120 min and at 5 minute intervals from t = 120 to t = 180 min.

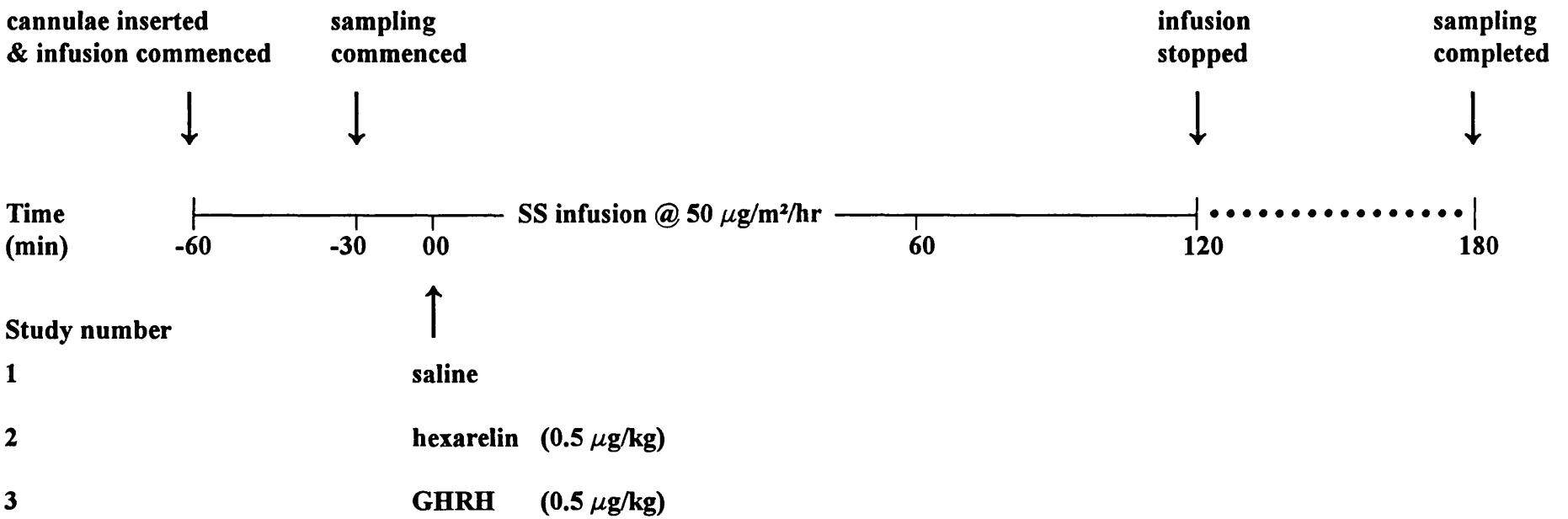


Figure 9b. Study design for set 3bii. SS (50 $\mu g/m^2/hr$) was infused intravenously from t = -60 to t = 120 min. At t = 0 min, an i.v. bolus of saline, hexarelin (0.5 μg/kg) or GHRH-(1-29)-NH_2 (0.5 μg/kg) was administered. The SS infusion was stopped at t = 120 min. Blood specimens were collected at 15 minute intervals from t = -30 to t = 120 min and at 5 minute intervals from t = 120 to t = 180 min.

4) Dose-response studies of the growth hormone, prolactin and cortisol response to hexarelin

The aim of this set of studies (set 4) was to establish a dose-response curve of the GH-releasing effect of hexarelin and to investigate whether the prolactin- and cortisol-releasing effects of hexarelin were dose-related. In addition, the effect of hexarelin on serum TSH, serum insulin and blood glucose was investigated. Ultimately, the question of whether a dose of hexarelin (alone or combined with GHRH) could bring about adequate GH release without the concomitant release of cortisol and prolactin was addressed.

Subjects

6 healthy adult males, aged 20.6 - 35.1 years with BMI 19.8 - 28.9 kg/m^2, were studied.

Study design

Following an overnight fast, two indwelling intravenous cannulae were inserted in the forearms at 0830 h (t = -60 minutes). One cannula was used for drug administration, and the other for collection of blood specimens. Subjects remained in a recumbent position throughout the study session. Blood samples for measurement of serum GH, PRL, cortisol, TSH, insulin and blood glucose were drawn at 15 minutes intervals from 0900 h (t = -30 minutes) to 1130 h (t = 120 minutes). At 0930 h (t = 0 minutes), a bolus of 0.9% saline, GHRH-(1-29)-NH_2 (1.0 μg/kg) or varying dosages of hexarelin (0.125 - 1.0 μg/kg) was administered intravenously (studies 1-8, Figure 10).

The studies were conducted in a random order with a washout period of at least 72 hours between each, though in the majority of cases the interval was seven days or more, and were completed by all 6 subjects. Following analysis of the data from studies 1-8 one further study was conducted using a combination of GHRH-(1-29)-NH_2 (1.0 μg/kg) and low dose hexarelin

(0.125 μg/kg) (study 9, Figure 10). These were completed by 4 out of the 6 subjects originally recruited.

Statistics

a) Curve fitting

Growth hormone data

The peak serum GH concentrations for each subject following the administration of intravenous saline and varying doses of hexarelin were used to construct the GH dose-response curve. The curve fitting function of the statistics package SPSS was used to determine the best fit model. The mean and SEM of the ED50 (the dose which would be expected to produce 50% of the maximal response) were obtained by curve fitting each subject's peak serum GH concentration following intravenous saline or varying doses of hexarelin, plotted against the dose administered.

The peak serum GH concentrations for each subject were also plotted against the $\log_{10}$ transformed hexarelin dose. Using the linear model of the curve fitting function of SPSS, a linear relationship was formulated.

Using the dot plot of the peak serum GH concentration against the $\log_{10}$ hexarelin dose, the responses of *each* individual subject to the various doses of hexarelin administered were drawn, thus illustrating each individual's peak response to each hexarelin dose. The purpose of this was to illustrate the subject effect on the peak GH response to hexarelin (would a high responder to a particular dose be a high responder to other doses?). The subject (and dose) effect on the peak GH response to varying doses of hexarelin was tested by two-way simple factorial ANOVA.

Prolactin and cortisol data

The data for serum PRL and cortisol concentrations were analysed on the basis of the maximum change following the administration of saline or varying doses of hexarelin compared with baseline values measured at t = 0 minutes. The rationale for using the *change* in hormone concentration as opposed to the absolute measured concentration is that, unlike GH, PRL and cortisol levels are detectable in the blood throughout the day and have a wide range of normal values with considerable inter-individual variability.

The data thus obtained were then used to construct the dose-response curves for each hormone. Curve fitting was possible for the PRL but not the cortisol data. The cortisol data were therefore presented as a dot-plot. The mean and SEM of the ED50 for the maximum change in serum PRL concentration were obtained by curve fitting each subject's maximum change in serum PRL concentration following intravenous saline or varying doses of hexarelin, plotted against the dose administered.

The maximum change in serum PRL and cortisol concentrations following the administration of saline or varying doses of hexarelin compared with baseline values measured at t = 0 minutes for each subject were also plotted against the $\log_{10}$ transformed hexarelin doses. Using the linear model of the curve fitting function of SPSS, linear relationships between the $\log_{10}$ dose and each of the maximum change in serum PRL and serum cortisol concentrations were formulated.

Using the dot plots of the maximum change in serum PRL and cortisol concentration against the $\log_{10}$ hexarelin dose, the responses of *each* individual subject to the various doses of hexarelin administered were drawn, thus illustrating each individual's maximum change in serum PRL and cortisol concentration following each hexarelin dose. The purpose of this was to illustrate the subject effect on the maximum change in serum PRL and cortisol following administration of various doses of hexarelin. The subject (and dose) effect was tested by two-way simple factorial ANOVA.

Comparison between the growth hormone- and prolactin-releasing activity of hexarelin

To test the relative activity of hexarelin on the somatotroph and the lactotroph, the maximum GH and PRL responses were plotted against the $\log_{10}$ hexarelin dose. To ensure uniformity of the data, comparison between the two hormonal responses was made on the basis of the maximum *change* in serum concentrations following the administration of saline or varying doses of hexarelin compared with baseline values measured at t = 0 minutes.

The linear relationship between the maximum change in serum GH from baseline at t = 0 minutes and the $\log_{10}$ doses of hexarelin was formulated (using SPSS) for the purposes of linear graphic presentation and comparison with the PRL data.

In addition, the hormonal responses were compared in molar terms. For GH, the latter was calculated by multiplying the change in serum concentration by 0.38 (to convert mU/L to μg/L) and dividing by 22000 (molecular weight of the fraction of GH measured by the Hybritech assay); for PRL, the change in serum concentration was multiplied by 0.3 (to convert mU/L to μg/L) and divided by 25000 (molecular weight of PRL) (PJ Pringle, personal communication). The maximum changes in serum GH and PRL in molar terms following the administration of each dose of hexarelin were then plotted (on the same graph, for the purposes of comparison) against the $\log_{10}$ hexarelin dose.

b) Glucose, Thyroid Stimulating Hormone and insulin data

The mean and SEM of the serum TSH, serum insulin and blood glucose concentrations at each sampling point following the intravenous bolus administration of hexarelin (1.0 μg/kg) (Figure 10, study 7) or, for comparison, saline (Figure 10, study 1) were plotted against the time of sample collection. Statistical comparison of the serum/blood concentration of each of the parameters before and after bolus administration in each study (each dose) as well as between studies (different doses) was performed using two-way ANOVA.

The mean and SEM of the maximum change in blood glucose and serum TSH and insulin

concentrations from baseline values at t = 0 minutes were calculated. The hexarelin dose where most change occurred, together with the mean and SEM of the maximum change are presented.

c) Data for the growth hormone releasing hormone and the combined growth hormone releasing hormone plus hexarelin studies

The mean and SEM for the peak serum GH concentration, the mean and SEM for the maximum percentage change in prolactin and cortisol concentrations and the mean and SEM for the maximum change in serum TSH, serum insulin and blood glucose concentrations from baseline values at t = 0 minutes were calculated and used for presentation.

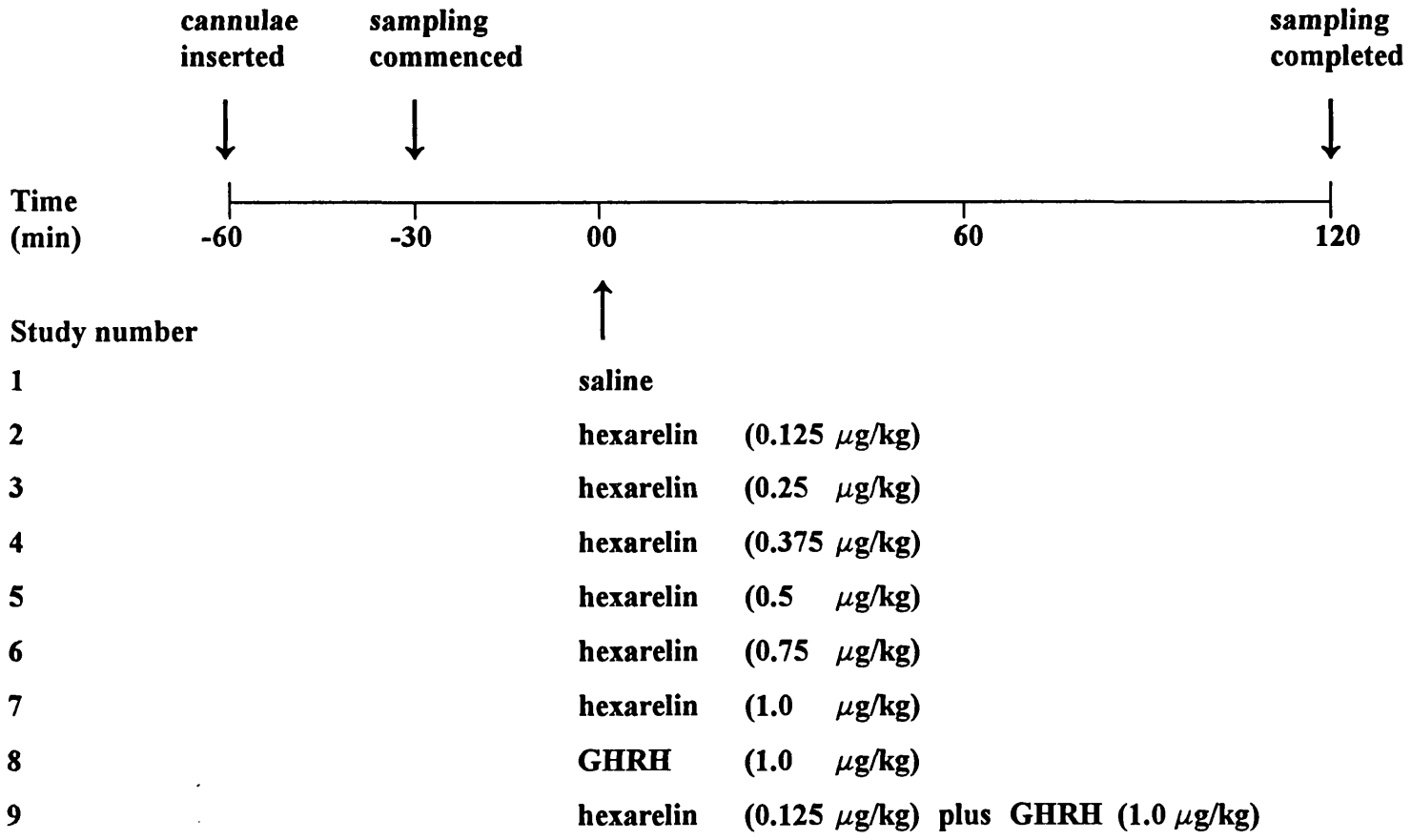


Figure 10. Study design for set 4. An i.v. bolus of saline (study 1), varying doses of hexarelin (studies 2-7), GHRH-(1-29)-NH_2 (1.0 μg/kg) (study 8), or hexarelin (0.125 μg/kg) plus GHRH-(1-29)-NH_2 (1.0 μg/kg) (study 9) was administered at t = 0 min. Blood specimens were collected at 15 minute intervals from t = -30 to t = 120 min.

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Section 3. Drugs, Dosages and Side-Effects

Hexarelin

Hexarelin was the only GHRP used in the studies and only by intravenous injection. Hexarelin vials containing 100 μg of lyophilized active ingredient were prepared and supplied by Europeptides, Argenteuil, France. The active ingredient was synthesized by Bachem, Budendorf, Switzerland. Sterile 0.9% saline was used for reconstitution and dilution.

The majority of the studies were conducted using an intravenous hexarelin dose of 1.0 μg of peptide per kg of body weight. The rationale for doing so was that this dose of hexarelin had been shown to produce near-maximal / maximal GH release whilst avoiding any side effects (Ghigo *et al.*, 1994a; Imbimbo *et al.*, 1994). Most of our studies were planned with a view to comparing the GH-releasing effect of intravenous hexarelin with that of GHRH. The dose of GHRH conventionally used in testing the readily releasable pool of GH from the pituitary is 1.0 μg of peptide per kg of body weight, a dose which is thought to be maximal/supramaximal for GH release when administered intravenously (Spoudeas *et al.*, 1994). Hence, it was decided to use a dose of 1.0 μg/kg for each of these peptides.

Doses of hexarelin other than 1.0 μg/kg were used in two groups of studies. The dose-response studies (set 4) entailed using varying dosages of hexarelin, ranging from 0.125 μg/kg through to 1.0 μg/kg. A smaller dose of hexarelin (0.5 μg/kg) was also used in a subset of the studies of SS withdrawal (set 3bii). The rationale for the latter was to avoid the potential depletion of the somatotroph stores of GH, which might have confounded the outcome in that particular set of studies. For similar reasons, a smaller dose of GHRH-(1-29)-NH_2 (0.5 μg/kg) was also used in that same set of studies.

Growth hormone releasing hormone

The analogue GHRH-(1-29)-NH_2, the synthetically produced N-terminal fragment of the endogenous peptides GHRH-(1-44)-NH_2 and GHRH-(1-40)-OH, was used. Vials (trade name: Groliberin) containing 500 μg of sterile powder were supplied by Pharmacia & Upjohn, Stockholm, Sweden. Sterile water was used for reconstitution and dilution.

Somatostatin

Natural somatostatin 1-14 was supplied by Ferring, Malmö, Sweden, in vials containing 250 μg of active ingredient in powder form. Sterile 0.9% saline was used for reconstitution and dilution.

The dose of SS(1-14) (50 μg / m^2 body surface area / hr) was based on previous studies showing adequate GH suppression (Hindmarsh *et al.*, 1991). In addition, a smaller dose of SS (20 $\mu g/m^2/hr$) was used for studies of SS tone and SS withdrawal.

Biosynthetic recombinant human growth hormone

The somatropin preparation Humatrope (Eli Lilly, Indiannapolis, USA) was used. Vials contained powder which provided 4 IU of GH following reconstitution with the supplied diluent. Sterile 0.9% saline was then used for diluting the reconstituted r-hGH to the required dose.

The dose of r-hGH used was 100 mU and was not adjusted for body size. This was based on previous studies (Brain *et al.*, 1993) which showed that doses of this magnitude were likely to result in serum GH concentrations comparable to physiological levels in adults.

Storage

Vials of hexarelin, GHRH-(1-29)-NH_2, r-hGH and SS were stored at 4^0 C until reconstitution.

Reconstitution procedures were carried out at room temperature. Following dissolution, the products were used within 4 hours, except for r-hGH which was used within 48 hours, having been stored at 4^0 C.

Side-effects

All subjects experienced transient facial flushing following the administration of GHRH-(1-29)-NH_2 but none reported any side effects following the administration of hexarelin, r-hGH or SS.

Section 4. Assays

Growth hormone

Serum GH concentrations were measured using an immunoradiometric assay (Hybritech Tandem-R hGH Kit, Hybritech, Liege, Belgium). The sensitivity of the assay was 0.5 mU/L; intra-assay coefficients of variation were 10.6%, 4.9%, 5.2%, 4.9% and 5.0% at serum GH concentrations of 1.4 mU/L, 3.5 mU/L, 14.4 mU/L, 26.4 mU/L and 99.4 mU/L, respectively; inter-assay coefficients of variation were 10.5%, 7.2%, and 5.4% at concentrations of 6.0 mU/L, 13.2 mU/L, and 33.3 mU/L, respectively. The standard used was HS2443E (NIH) which had been recalibrated to mU/L (1 ng/ml = 2.6 mU/L) with the International Standard 80/505. The assay did not cross-react with PRL.

Prolactin

Serum PRL concentrations were measured using an immunoradiometric assay (NETRIA, St. Bartholomew's Hospital, London, UK). The sensitivity of the assay was 10 mU/L; intra-assay coefficients of variation were 2.5%, 1.4%, and 2.6% at serum PRL concentrations of 165 mU/L, 562 mU/L and 1159 mU/L, respectively; inter-assay coefficients of variation were 8.3%, 5.1% and 7.9% at concentrations of 173 mU/L, 506 mU/L and 1103 mU/L, respectively. The assay did not cross-react with GH.

Cortisol

Serum cortisol concentrations were measured using a solid phase radioimmunoassay (Coat-A-Count, Diagnostic Products Corp., Los Angeles, CA, USA). The sensitivity of the assay was 5.5 nmol/L; intra-assay coefficients of variation were 5.7%, 3.1% and 2.6% at serum cortisol concentrations of 27.6 nmol/L, 96 nmol/L and 552 nmol/L, respectively; inter-assay coefficients of variation were 6.3%, and 4.5% at concentrations of 138 nmol/L and 276

nmol/L, respectively.

Thyroid Stimulating Hormone

Serum TSH concentrations were measured using a solid phase immunoradiometric assay kit (NETRIA, St. Bartholomew's Hospital, London, UK). The sensitivity of the assay was 0.2 mU/L; intra-assay coefficients of variation were 3.1%, 1.6%, and 1.9% at serum TSH concentrations of 1.5 mU/L, 14.0 mU/L and 23.4 mU/L, respectively; inter-assay coefficients of variation were 10.2%, 3.8% and 3.7% at serum TSH concentrations of 1.8 mU/L, 16.8 mU/L and 25.9 mU/L, respectively. The standards used were calibrated against the International Reference Preparation, MRC 80/558.

Insulin

Serum insulin concentrations were measured using a commercial radioimmunoassay (Diagnostic Systems Laboratories, Webster, Texas, USA). The sensitivity of the assay was 1.5 mU/L; intra-assay coefficients of variation were 8.2%, 4.8%, and 6.3% at serum insulin concentrations of 4.8 mU/L, 17.6 mU/L and 54.6 mU/L, respectively; inter-assay coefficients of variation were 11.2%, 9.5% and 6.5% at serum insulin concentrations of 4.9 mU/L, 16.6 mU/L and 49.7 mU/L, respectively. The standards used were calibrated against the WHO Reference Preparation, 83/500.

Glucose

Whole blood glucose concentrations were measured using the glucose oxidase method by means of a Yellow Springs Industrial Analyzer model 23 (Yellow Springs, Ohio, USA). The intra-assay coefficients of variation were 0.8 and 0.9% at concentrations of 4.0 and 8.0 mmol/L, respectively.

Insulin like growth factor-1 (IGF-1)

Serum IGF-1 concentrations were measured using a polyclonal radioimmunoassay. The sensitivity of the assay was 13.9 μg/L; intra-assay coefficients of variation were 11.3%, 6.5%, and 4.7% at concentrations of 45.5 μg/L, 243.5 μg/L, and 698.9 μg/L, respectively, and inter-assay coefficients of variation were 10.5%, 12.1% and 5.1% at concentrations of 75.2 μg/L, 196.0 μg/L, and 698.9 μg/L, respectively.

All samples, except those for blood glucose measurement, were spun immediately following the end of each sampling session. Serum was separated and stored at -20^0 C until assayed. Blood glucose concentrations were measured using Yellow Springs Industrial Analyzer immediately following each study session.

Section 5. Statistics

The statistics software programme SPSS for Windows (Release 6.0) was used in the analysis.

Comparison of group means

a) Wilcoxon matched pairs signed rank sum test

This is a non-parametric method used for comparing two groups of paired data. Paired data arise when the same individuals are studied more than once, usually in different circumstances. This was the case in the studies reported in this thesis. Data can be paired also if two different but individually matched groups of subjects have been studied.

b) Mann-Whitney test

This is a non-parametric method used for comparing data from two independent groups.

c) One-way analysis of variance (one-way ANOVA)

This is a method used to compare the means of three or more sets of data, either from a single sample or from independent samples. It thus represents an extension of the t-tests (for parametric data) or the Mann-Whitney test (for non-parametric data) to situations involving more than two groups defined by a single factor. The principle behind analysis of variance is to subdivide the total variability of a set of data into components due to different sources of variation. This is done by assessing how much of the overall variation in the data is attributable to the differences *between* the group means, and comparing it with the amount attributable to differences between individuals *within* each group. The comparison takes the general form of an F test to compare variances {the ratio of the variance estimated from the means of the groups (the *between* group variation) and the variance between the individuals

within the groups}. If the F value achieves the level of significance required, then differences between individual groups can be investigated, unless certain comparisons were intended in advance of the analysis.

It is possible to compare each pair of means in turn, but such multiple significance testing gives a high probability of finding a significant difference just by chance. This problem of multiple comparisons is dealt with by applying an *a posteriori* test (e.g. Newman-Keuls) which aims at controlling the overall Type 1 error (finding a significant difference when there is no real difference) rate at no more than 5%.

After carrying out the analysis of variance the variation of the individual observations around the mean of their sample is examined (the difference between the two values is called a "residual"). If the distribution of the residuals is not Normal, the data may need to be transformed or reanalysed using a non-parametric test. The Kruskal-Wallis test, the general form of the non-parametric Mann-Whitney test, is used for comparison of means of three or more sets of data.

d) Two-way analysis of variance on repeated measures (two-way ANOVA)

In contrast to one-way ANOVA, two-way ANOVA is used when the groups to be compared are defined by two factors. It thus allows the simultaneous comparison of the effects of two factors on the response variable and in addition make it possible to investigate their interactions. Repeated measures design are used when the subjects act as their own controls and are observed at a number of different time points.

The principle behind two-way ANOVA is similar to that of one-way ANOVA, though, naturally, is more complicated. Similarly, the significance (F) test is performed and an *a posteriori* test is applied (e.g. Scheffé) as described for one-way ANOVA. The non-parametric Friedman's two-way ANOVA is used for data sets which do not fulfil the assumptions of the parametric method.

Regression

a) Linear regression

This method is used to investigate the linear association between two continuous variables. The correlation coefficient gives a measure of the closeness of an association, while the linear regression gives an equation of the straight line that best describes it and thus enables the prediction of one variable from the other. Log transformation of the data frequently allows clearer demonstration of the linear relationship.

The linear regression equation was used in the GH feedback studies to calculate the serum GH concentration *immediately* following exogenous r-hGH administration. The measured serum GH concentrations were natural log transformed and plotted against time of sample collection. The relationship between time and the transformed data was described by linear regression. The serum GH concentration at the point t = 0 minutes was represented by the anti-$\log_e$ of the intercept of the regression.

The linear regression equation was also used in the analysis of dose-response studies to describe the linear relationships between the $\log_{10}$ hexarelin doses and the maximum GH, PRL and cortisol responses. Where a non-linear equation was obtained (e.g. quadratic), stepwise linear regression analysis was performed to test the significance of the non-linear components of the equation.

b) Polynomial (curvi-linear) regression

This method is used when the relation between two variables is curved. The curve fitting function of the above-mentioned statistics software programme was used in the analysis of the dose-response studies. The cubic model of curve estimation was used to construct the best fit curves.

Analysis of GH secretion

Deconvolution analysis

This is a technique used to assess the contribution of hormone secretion to the observed serum concentration. The technique requires prior knowledge of the mode of secretion of a hormone or, alternatively, a measure of the half-life of that hormone within the circulation. A deconvolution analysis model to calculate pituitary GH secretion rates based on the methodology of Turner *et al* (1971) and using a variable half-life of GH has been described by Hindmarsh *et al* (1990). The method involves sequential subtraction of the half-life declination per unit time from the data.

Deconvolution analysis was used to determine the GH secretion rates in the studies of repeated bolus administration of hexarelin, GHRH-(1-29)-NH_2 or hexarelin plus GHRH-(1-29)-NH_2.

CHAPTER 3

RESULTS

Results are presented under four main sections. Each section deals with a set of studies (sets 1-4) described in Chapter 2 (Methods). Reference is made frequently to the Figures which illustrate the protocol design of each set of studies. These Figures are shown in the 'Studies' Section in Chapter 2.

Section 1. The Effect of Single and Repeated Doses of Hexarelin, Growth Hormone Releasing Hormone and Hexarelin plus Growth Hormone Releasing Hormone on Growth Hormone Secretion

Serum growth hormone concentrations and growth hormone secretion rates

In this set of studies, where two boluses of secretagogue(s) were administered within a short interval (60 or 120 minutes apart), the data were analysed and presented mainly in terms of GH secretion rates (rather than actual serum GH concentrations) in order to avoid errors associated with the interpretation of serum GH concentrations on the descending limb of a GH pulse. With the exception of two aspects (where actual serum GH concentrations are used for presentation - see below), the data are presented as GH secretion rates in mU/min. The latter were calculated using the deconvolution method of Hindmarsh *et al* (1990) which requires prior knowledge of the half-life of GH and takes into account the volume of distribution based on total body weight (Hindmarsh *et al.*, 1989).

Serum growth hormone concentrations

a) Pattern of the growth hormone response

To illustrate the pattern of response to intravenous administration of a single bolus of saline, hexarelin, GHRH-(1-29)-NH_2 or both together (Figure 6, studies 1, 2, 5 and 8, respectively), mean and SEM of the actual serum GH concentrations measured at 15 minute intervals following administration of the secretagogue(s) were calculated and are shown in Figure 11. It can be seen from Figure 11 that the GH levels peaked before t = 60 minutes and returned to baseline by t = 180 minutes. At the time of administration of the second bolus (t = 60 or t = 120 minutes), serum GH levels were still elevated but decreasing, confirming the need to

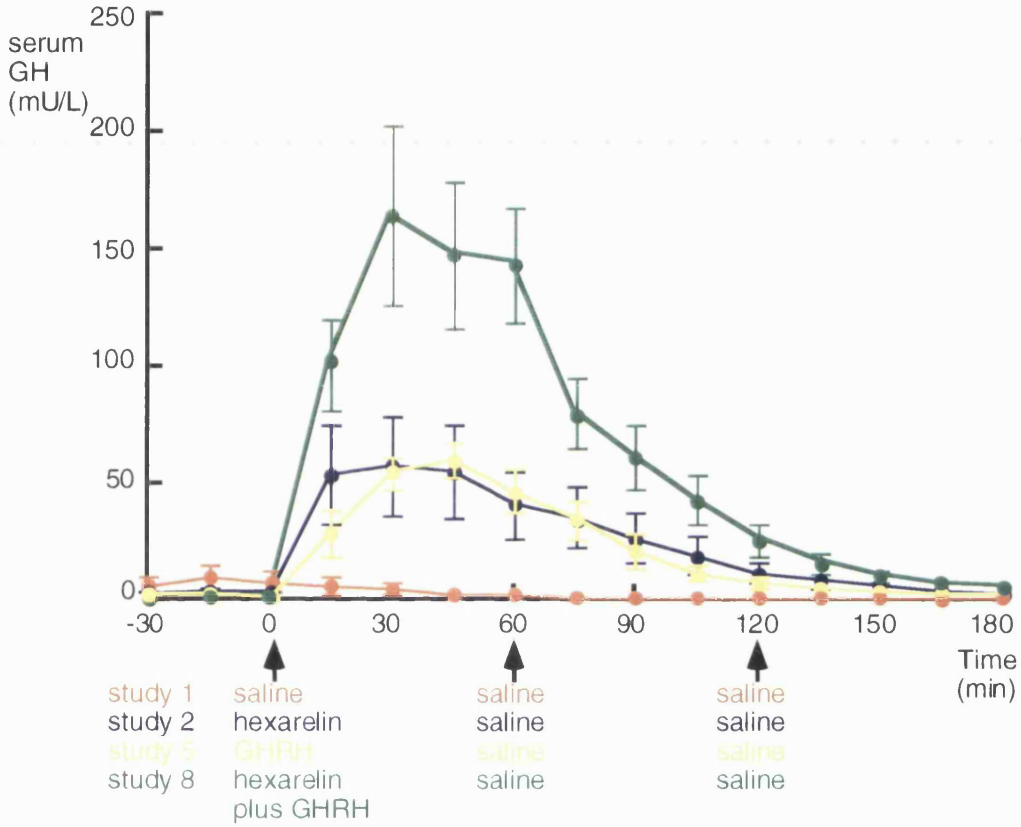


Figure 11. Serum GH concentration profiles following a single intravenous bolus of saline, hexarelin, GHRH-(1-29)-NH_2 or hexarelin plus GHRH-(1-29)-NH_2 in six healthy subjects. Secretagogue doses of 1.0mg/kg were used throughout. Data shown as mean ± SEM.

deconvolute the data.

The mean, SEM and median times for the peak GH response following i.v. bolus administration of hexarelin, GHRH-(1-29)-NH_2 or hexarelin plus GHRH-(1-29)-NH_2 are shown in Table 12. There was no significant difference in the timing of the GH peak following secretagogue(s) administration (ANOVA $p > 0.05$).

Table 12. Time (minutes) to peak serum GH concentration following intravenous bolus administration of hexarelin, GHRH-(1-29)-NH_2 or hexarelin plus GHRH-(1-29)-NH_2.

Bolus	Time to peak (median)	Time to peak (mean)	SEM
hexarelin	45 minutes	37.5 minutes	3.0
GHRH-(1-29)-NH_2	30 minutes	33.3 minutes	4.3
hexarelin plus GHRH-(1-29)-NH_2	45 minutes	37.5 minutes	2.5

b) Magnitude of the growth hormone response

The magnitude of the peak serum GH response to a single bolus of hexarelin, GHRH-(1-29)-NH_2 and hexarelin plus GHRH-(1-29)-NH_2 (pooled data from studies 2-10, Figure 6) is shown in Figure 12. The peak serum GH response to hexarelin was significantly greater than that to GHRH-(1-29)-NH_2 (hexarelin peak = 85.5 ± 10.5 (mean ± SEM) mU/L; GHRH-(1-29)-NH_2 peak = 39.9 ± 9.2 mU/L; Mann-Whitney $p = 0.001$). The peak serum GH concentration following the combined administration of hexarelin plus GHRH-(1-29)-NH_2 was 194.1 ± 23.9 mU/L, which, in the words of the originator of GHRPs, could be described as "massive" (Bowers, 1993b).

c) Synergistic action of hexarelin plus GHRH-(1-29)-NH_2

The GH response to coadministration of hexarelin plus GHRH-(1-29)-NH_2 was greater than the additive effect of the two secretagogues (the arithmetic sum of the peak serum GH concentrations following their isolated administration), as shown in Figure 12 (hexarelin plus GHRH-(1-29)-NH_2 peak = 194.1 ± 23.9 mU/L; arithmetic sum = 125.5 ± 13.0 mU/L; Wilcoxon $p < 0.001$). This confirmed that hexarelin plus GHRH-(1-29)-NH_2 was synergistic for GH release.

The data for the Figure 12 are given in Appendix 1.

d) Intra- and inter-individual variability in the peak GH response to hexarelin and GHRH-(1-29)-NH_2

Intra-individual variability

Six subjects, who had undergone four or more study sessions (from the entire four sets of studies) in which only hexarelin or GHRH-(1-29)-NH_2 had been administered at a dose of 1.0 μg/kg, were identified. The coefficients of variance of the peak GH responses to hexarelin and GHRH-(1-29)-NH_2 in these individuals (intra-individual variability) were 43.4 ± 14.0 % (mean ± SD) and 69.6 ± 17.2 %, respectively.

Inter-individual variability

Six study sessions, in which only hexarelin or GHRH-(1-29)-NH_2 had been administered at a dose of 1.0 μg/kg, were identified. The coefficients of variance of the peak GH responses to hexarelin and GHRH-(1-29)-NH_2 for the different study sessions (inter-individual variability) were 46.1 ± 13.9 % (mean ± SD) and 74.4 ± 20.9 %, respectively.

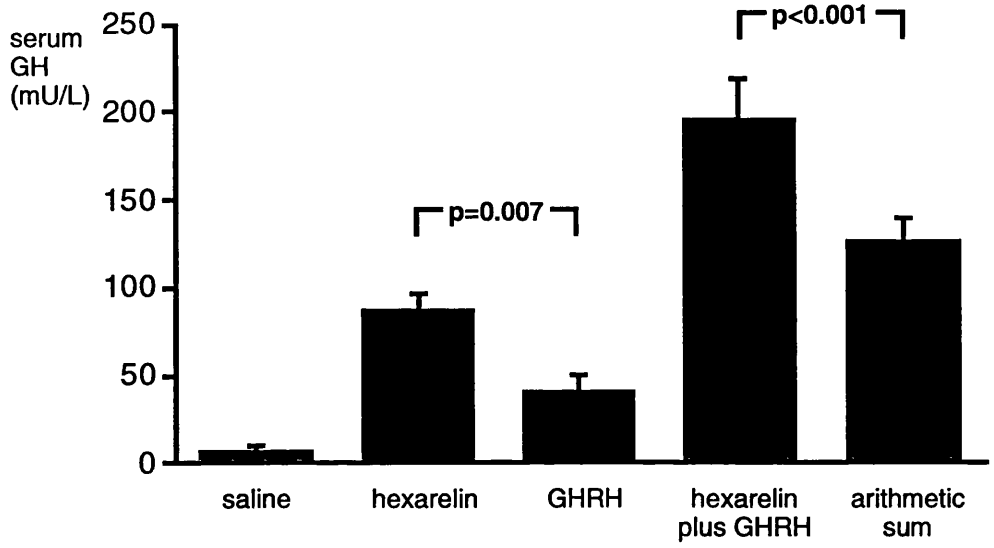


Figure 12. Pooled peak serum GH concentrations following intravenous boluses at t = 0 min of saline (Figure 6, study 1), hexarelin (Figure 6, studies 2, 3 and 4), GHRH-(1-29)-NH_2 (Figure 6, studies 5, 6 and 7) or hexarelin plus GHRH-(1-29)-NH_2 (Figure 6, studies 8, 9 and 10) in six healthy subjects. The additive effect of hexarelin and GHRH-(1-29)-NH_2 (the arithmetic sum of the peak serum GH concentrations following their isolated administration) is also shown for comparison. Data shown as mean + SEM.

Growth hormone secretion rates

a) First bolus saline, hexarelin, GHRH-(1-29)-NH_2 or hexarelin plus GHRH-(1-29)-NH_2

Although the data for the first boluses have already been reported above in terms of actual GH concentrations, they are presented here in terms of GH secretion rates in order to simplify the comparison and interpretation of the data for the second boluses, which were analysed more appropriately in terms of GH secretion rates.

The peak GH secretion rate following the first intravenous injection of hexarelin was greater than that following the first administration of GHRH-(1-29)-NH_2 (hexarelin peak = 265.8 ± 39.2 mU/min; GHRH-(1-29)-NH_2 peak = 131.0 ± 33.9 mU/min; two-way ANOVA $p < 0.001$), and was greatest following the administration of both secretagogues (hexarelin plus GHRH-(1-29)-NH_2 peak = 628.4 ± 84.0 mU/min; two-way ANOVA $p < 0.001$) (Figure 13).

The peak GH secretion rate following hexarelin plus GHRH-(1-29)-NH_2 was significantly greater than the arithmetic sum of the peak GH secretion rates following their isolated administration (hexarelin plus GHRH-(1-29)-NH_2 peak = 628.4 ± 84.0 mU/min; arithmetic sum = 396.8 ± 49.0 mU/min; Wilcoxon $p = 0.001$) (Figure 13).

b) Second bolus saline, hexarelin, GHRH-(1-29)-NH_2 or hexarelin plus GHRH-(1-29)-NH_2

i) Second bolus secretagogue(s) *vs* second bolus saline

Second bolus at t = 60 minutes

The administration of a second intravenous bolus of hexarelin, GHRH-(1-29)-NH_2, or hexarelin plus GHRH-(1-29)-NH_2 60 minutes after the first resulted in further GH secretion, which was significantly greater than that seen after saline administration {(60 minute boluses: hexarelin peak = 222.1 ± 58.3 mU/min; saline peak = 38.7 ± 19.0 mU/min: two-way ANOVA $p = 0.02$, GHRH-(1-29)-NH_2 peak = 83.4 ± 32.8 mU/min;

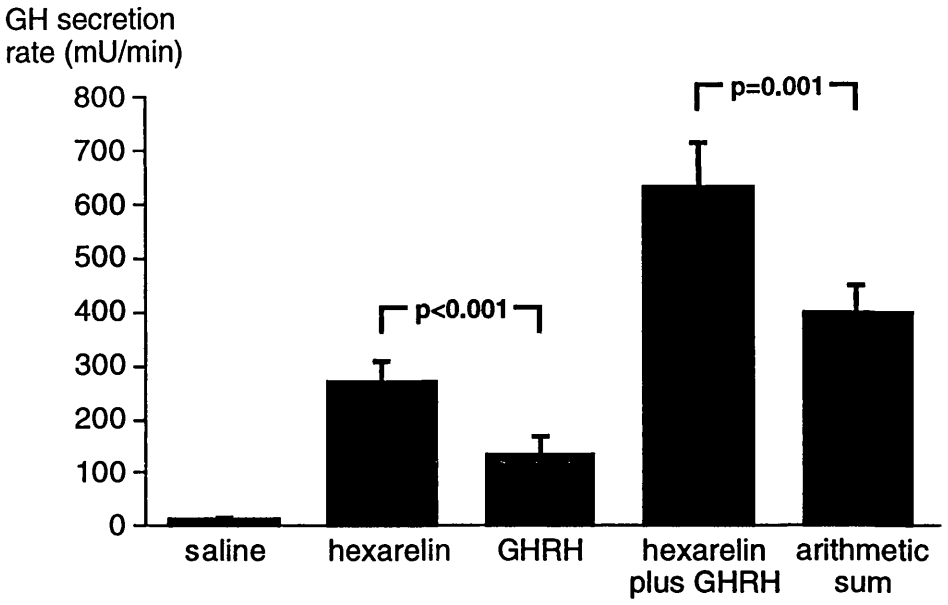


Figure 13. Pooled peak GH secretion rates following intravenous boluses at t = 0 min of saline (Figure 6, study 1), hexarelin (Figure 6, studies 2, 3 and 4), GHRH-(1-29)-NH_2 (Figure 6, studies 5, 6 and 7), and hexarelin plus GHRH-(1-29)-NH_2 (Figure 6, studies 8, 9 and 10), and the arithmetic sum of the peak GH secretion rates following the isolated administration of each secretagogue. Data shown as mean + SEM.

saline peak = 59.0 ± 29.5 mU/min: two-way ANOVA p = 0.002, hexarelin plus GHRH-(1-29)-NH_2 peak = 469.9 ± 77.8 mU/min; saline peak = 68.6 ± 30.4 mU/min: two-way ANOVA p = 0.03).

Second bolus at t = 120 minutes

Similarly, the administration of a second intravenous bolus of hexarelin, GHRH-(1-29)-NH_2, or hexarelin plus GHRH-(1-29)-NH_2 120 minutes after the first resulted in further GH secretion, which was significantly greater than that seen after saline administration {(120 minute boluses: hexarelin peak = 124.9 ± 25.6 mU/min; saline peak = 1.4 ± 0.5 mU/min: two-way ANOVA p = 0.01, GHRH-(1-29)-NH_2 peak = 126.3 ± 61.1 mU/min; saline peak = 7.9 ± 3.4 mU/min: two-way ANOVA p = 0.02, hexarelin plus GHRH-(1-29)-NH_2 peak = 265.1 ± 62.7 mU/min; saline peak = 9.7 ± 3.9 mU/min: two-way ANOVA p = 0.03)}.

ii) Second bolus secretagogue(s) *vs* first bolus secretagogue(s)

hexarelin boluses

The peak GH secretion rates following the first hexarelin bolus (at t = 0 min) and the *corrected* (for explanation, see Chapter 2, Studies set 1, *statistics*) peak GH secretion rates following the second hexarelin bolus (at t = 60 or t = 120 min) are shown in Figure 14. The *corrected* peak GH secretion rate at t = 120 minutes was significantly lower than that following the first bolus (t = 120 minutes bolus *corrected* peak = 123.5 ± 25.3 mU/min; t = 0 minutes bolus peak = 331.0 ± 108.1 mU/min: Wilcoxon p = 0.03). Although the *corrected* peak secretion rate following the second hexarelin bolus at t = 60 minutes was lower than that following the first bolus, this reduction was not statistically significant (t = 60 minutes bolus *corrected* peak = 183.4 ± 44.2 mU/min; t = 0 minutes bolus peak = 263.7 ± 40.6 mU/min: Wilcoxon p = NS).

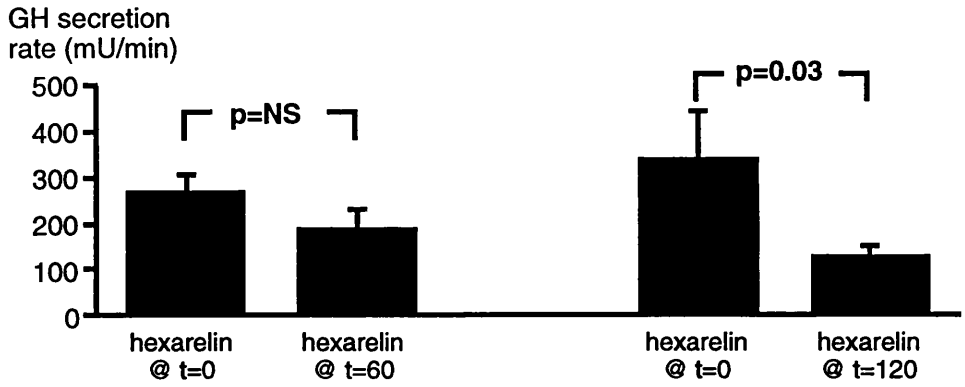


Figure 14. *Actual* peak GH secretion rates following an intravenous bolus of hexarelin at t = 0 min and *corrected* (for explanation, see Methods, Studies set 1, *statistics*) peak GH secretion rates following an intravenous bolus of hexarelin at t = 60 min (Figure 6, study 3). Similarly, the peak GH secretion rates following hexarelin at t = 0 min and t = 120 min (Figure 6, study 4) are shown. Data shown as mean + SEM.

hexarelin plus GHRH-(1-29)-NH_2 boluses

A similar phenomenon to the above was seen following the administration of the second bolus of hexarelin plus GHRH-(1-29)-NH_2, where the peak GH secretion rate after the t = 120 minutes bolus was significantly lower than that after the first, whereas that after the t = 60 minutes bolus was not significantly lower (t = 120 minutes bolus *corrected* peak = 255.4 ± 61.0 mU/min; t = 0 minutes bolus peak = 754.7 ± 200.6 mU/min: Wilcoxon p = 0.03, t = 60 minutes bolus *corrected* peak = 401.3 ± 83.3 mU/min; t = 0 minutes bolus peak = 583.0 ± 115.2 mU/min: Wilcoxon p = NS) (Figure 15).

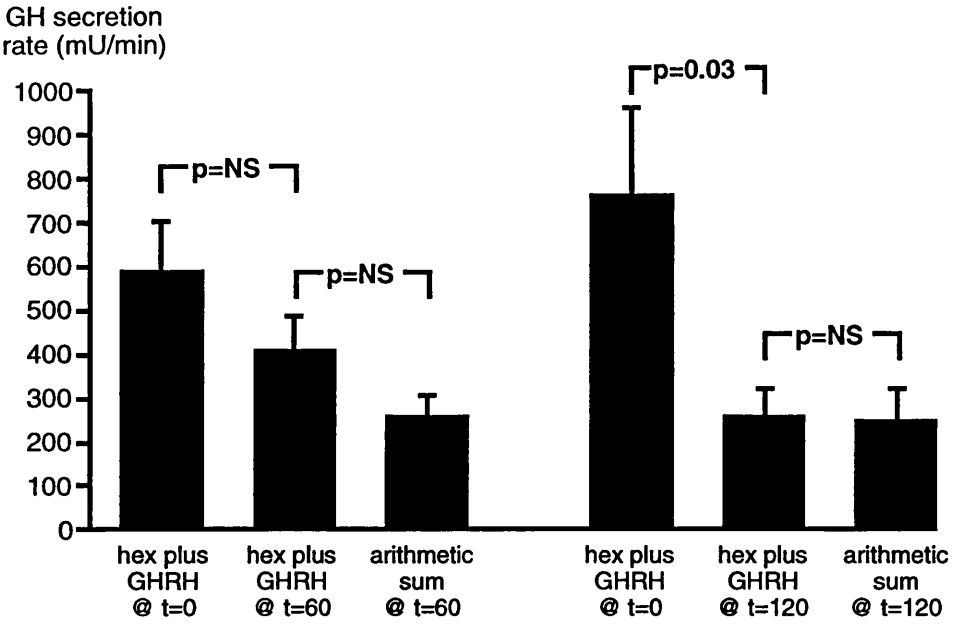
GHRH-(1-29)-NH_2 boluses

The administration of a second bolus of GHRH-(1-29)-NH_2, irrespective of whether it was given 60 minutes or 120 minutes after the first, resulted in similar GH secretion rates (t = 120 minutes bolus *corrected* peak = 118.4 ± 60.1 mU/min; t = 0 minutes bolus peak = 95.8 ± 29.2 mU/min: Wilcoxon p = NS, t = 60 minutes bolus *corrected* peak = 70.0 ± 34.9 mU/min; t = 0 minutes bolus peak = 79.6 ± 29.4 mU/min: Wilcoxon p = NS) (Figure 16).

hexarelin plus GHRH-(1-29)-NH_2 boluses - synergistic / additive effect

The peak GH secretion rate following the second bolus of hexarelin plus GHRH-(1-29)-NH_2 given 60 or 120 minutes after the first was not significantly different from the arithmetic sum (additive effect) of the peak GH secretion rates following their isolated administration given 60 or 120 minutes after the first respectively, indicating loss of synergistic activity (t = 60 minutes hexarelin plus GHRH-(1-29)-NH_2 bolus *corrected* peak = 401.3 ± 83.3 mU/min; arithmetic sum = 253.5 ± 48.9 mU/min: Wilcoxon p = NS, t = 120 minutes hexarelin plus GHRH-(1-29)-NH_2 bolus *corrected* peak = 255.4 ± 60.98 mU/min; arithmetic sum = 241.9 ± 74.1 mU/min: Wilcoxon p = NS). These data are shown in Figure 15.

The data for Figures 13-16 are given in Appendix 2.



(hex = hexarelin)

Figure 15. *Actual* peak GH secretion rates following an intravenous bolus of hexarelin plus GHRH-(1-29)-NH_2 at t = 0 min and *corrected* (for explanation, see Methods, Studies set 1, *statistics*) peak GH secretion rates following an intravenous bolus of hexarelin plus GHRH-(1-29)-NH_2 at t = 60 min (Figure 6, study 9). Similarly, the peak GH secretion rates following hexarelin plus GHRH-(1-29)-NH_2 at t = 0 min and t = 120 min (Figure 6, study 10) are shown. The arithmetic sums of the *corrected* peak GH secretion rates following the isolated administration of each secretagogue at t = 60 min and t = 120 min (Figure 6, studies 3 and 6, and studies 4 and 7, respectively) are also shown. Data shown as mean + SEM.

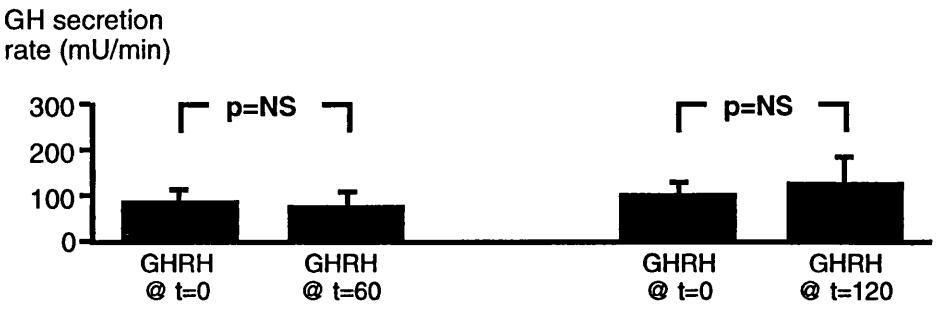


Figure 16. *Actual* peak GH secretion rates following an intravenous bolus of GHRH-(1-29)-NH_2 at t = 0 min and *corrected* (for explanation, see Methods, Studies set 1, *statistics*) peak GH secretion rates following an intravenous bolus of GHRH-(1-29)-NH_2 at t = 60 min (Figure 6, study 6). Similarly, the peak GH secretion rates following GHRH-(1-29)-NH_2 at t = 0 min and t = 120 min (Figure 6, study 7) are shown. Data shown as mean + SEM.

Summary

1. On a weight basis, the GH-releasing activity of hexarelin was greater than that of GHRH-(1-29)-NH_2. The issue of comparison between the two secretagogues on a molar basis is discussed in Chapter 4, Section 1.

2. The coadministration of hexarelin plus GHRH-(1-29)-NH_2 was synergistic for GH release.

3. The time taken to reach peak serum GH concentrations following intravenous bolus administration of hexarelin, GHRH-(1-29)-NH_2 or hexarelin plus GHRH-(1-29)-NH_2 was similar.

4. A second intravenous bolus of hexarelin, GHRH-(1-29)-NH_2 or hexarelin plus GHRH-(1-29)-NH_2 administered 60 or 120 minutes after the first resulted in further significant GH release.

5. The GH response to a second bolus of hexarelin or hexarelin plus GHRH-(1-29)-NH_2 administered 120 minutes, but not 60 minutes, after the first was significantly lower than that following the first bolus. The GH response to a second bolus of GHRH-(1-29)-NH_2 was similar in magnitude to the first and was unaffected by the time interval used in this set of studies.

6. The synergistic activity of hexarelin plus GHRH-(1-29)-NH_2 was not observed following the second bolus administration of the secretagogues regardless of the time interval between the two boluses.

7. There is less intra- and inter-individual variability in the peak GH response to hexarelin compared with the peak GH response to GHRH-(1-29)-NH_2.

Section 2. The Effect of Growth Hormone Feedback on Hexarelin-Induced Growth Hormone Release

Growth hormone feedback - representative profile from one subject

Figure 17 shows a representative serum GH concentration profile from a 26 year old subject (initials S.D., BMI 25.5 kg/m^2) who underwent this set of studies (protocol design shown in Figure 7). A large GH response was observed following the administration at t = 90 minutes of an intravenous bolus of hexarelin (1.0 μg/kg) or GHRH-(1-29)-NH_2 (1.0 μg/kg). This response, which was greater following hexarelin compared to GHRH-(1-29)-NH_2, was attenuated by the prior administration at t = 0 minutes of an intravenous bolus of r-hGH (100 mU), which itself resulted in a small rise in serum GH concentration. The degree of attenuation of the GH response in this individual was greater for GHRH-(1-29)-NH_2 than for hexarelin.

Growth hormone feedback - group data

The group data are illustrated in Figure 18, which shows the mean peak serum GH response to an intravenous injection at t = 90 minutes of hexarelin or GHRH-(1-29)-NH_2 following pretreatment at t = 0 minutes with intravenous r-hGH or saline.

a) Growth hormone response to hexarelin or GHRH-(1-29)-NH_2

The response to hexarelin administration was significantly greater than that following GHRH-(1-29)-NH_2, irrespective of whether pretreatment was with saline (hexarelin peak = 122.6 ± 28.3 mU/L (mean ± SEM); GHRH-(1-29)-NH_2 peak = 45.5 ± 14.6 mU/L: Mann-Whitney $p < 0.04$) or r-hGH (hexarelin peak = 83.1 ± 12.6 mU/L; GHRH-(1-29)-NH_2 peak = 26.2 ± 12.1 mU/L: Mann-Whitney $p < 0.02$) (Figure 18).

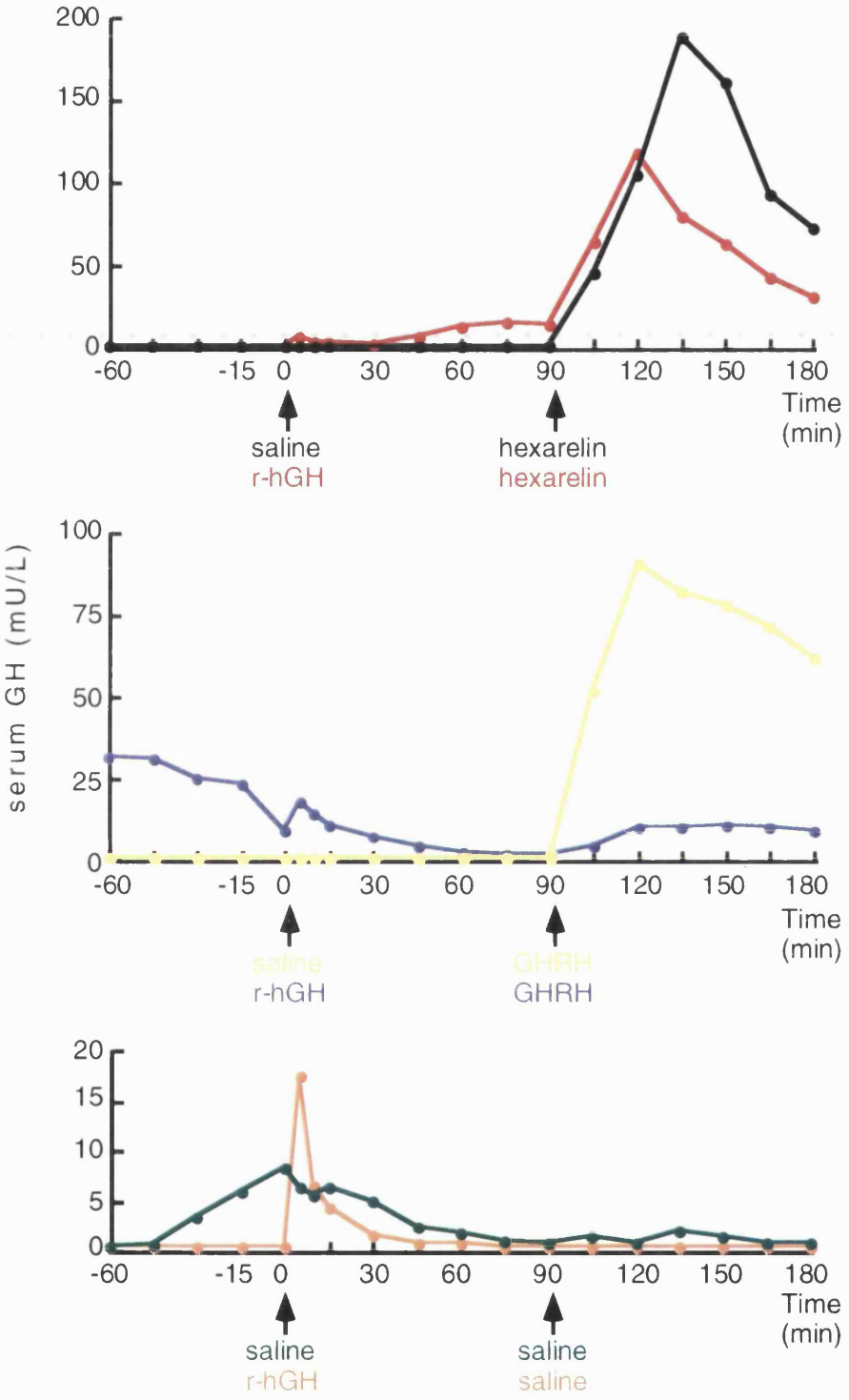


Figure 17. Representative serum GH concentration profiles from a 26 year old subject showing the attenuating effect of prior administration of intravenous r-hGH (100 mU) on the GH response to intravenous hexarelin (1.0mg/kg) (top panel) and GHRH-(1-29)-NH_2 (1.0mg/kg) (middle panel). Control studies are shown in the bottom panel.

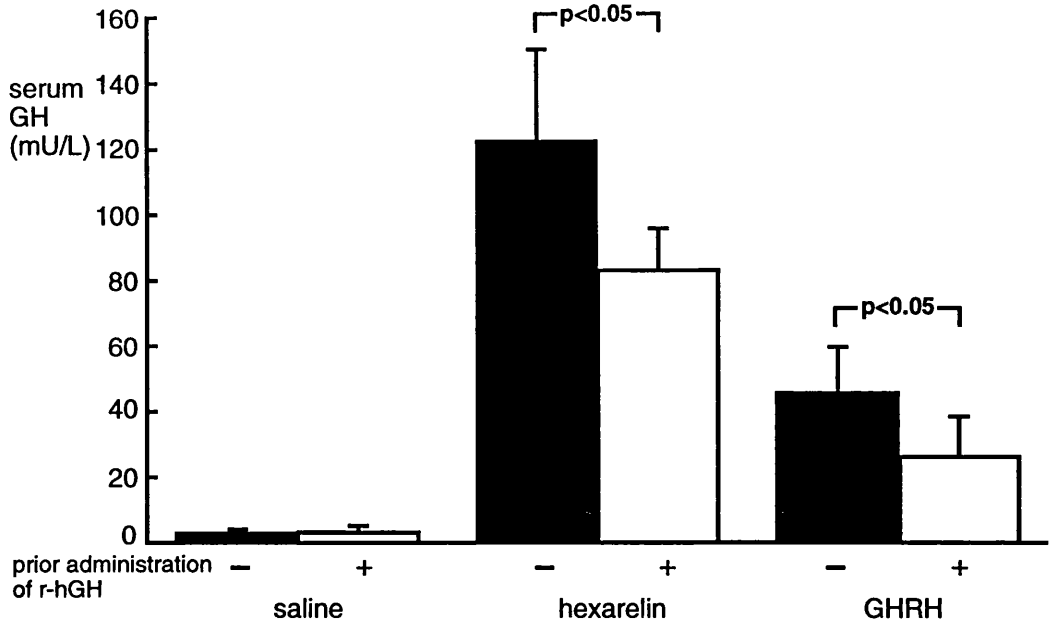


Figure 18. The effect of prior administration of intravenous saline or r-hGH (100 mU) on the peak serum GH response to a subsequent intravenous bolus injection of saline, hexarelin (1.0μg/kg) or GHRH-(1-29)-NH_2 (1.0μg/kg), given 90 minutes later. Prior administration of r-hGH at t = 0 min is denoted by the positive sign. Data shown as mean + SEM.

b) The effect of prior administration of recombinant human growth hormone

Prior administration of 100 mU r-hGH led to a significant reduction in the peak serum GH response to hexarelin (saline pretreatment, peak = 122.6 ± 28.3 mU/L; r-hGH pretreatment, peak = 83.1 ± 12.6 mU/L: Wilcoxon $p < 0.05$). Similarly, prior administration of r-hGH resulted in a reduction in the peak serum GH response to GHRH-(1-29)-NH_2 (saline pretreatment, peak = 45.5 ± 14.6 mU/L; r-hGH pretreatment, peak = 26.2 ± 12.1 mU/L: Wilcoxon $p < 0.05$) (Figure 18).

The percentage reduction in the GH response resulting from pretreatment with r-hGH was determined by calculating the reduction in each individual's peak GH response rather than the reduction in the overall mean peak values. The percentage reduction in response to hexarelin was less than that to GHRH-(1-29)-NH_2 (hexarelin 7.4 ± 23.7%; GHRH-(1-29)-NH_2 42.0 ± 15.1%). This difference was not statistically significant (Wilcoxon $p = 0.3$).

c) Serum growth hormone concentrations following intravenous recombinant human growth hormone

The serum GH concentrations *immediately* following the intravenous administration of 100 mU of r-hGH were calculated on an individual basis by the anti-$\log_e$ of the intercept of the linear regression which described the relationship between the natural log transformed measured serum GH concentrations and time of sample collection. Administration of 100 mU r-hGH produced similar GH levels in each of the studies (study 4 = 10.5 ± 0.5 mU/L; study 5 = 9.5 ± 1.6 mU/L; study 6 = 12.0 ± 1.9 mU/L: one-way ANOVA p = NS) and all were within the physiological range.

These data, and those shown in Figures 17 and 18 are given in Appendix 3.

d) Insulin-like growth factor-1 levels

Serum IGF-1 concentrations just before the bolus injections of either saline or 100 mU r-hGH (at t = 0 minutes) were similar in all studies, as were values after injection (at t = 90 minutes) {studies 1-3 (see Figure 7): pre saline = 241.6 ± 23.8 μg/L, post saline = 233.6 ± 19.8 μg/L; studies 4-6 (see Figure 7): pre r-hGH = 221.8 ± 37.6 μg/L, post r-hGH = 223.7 ± 17.8 μg/L: one-way ANOVA p = NS).

IGF-1 data are given in appendix 4.

The effect of hexarelin on prolactin, cortisol and Thyroid Stimulating Hormone release

As discussed in the Chapter 2, blood specimens collected during the execution of this set of studies were used for the preliminary investigation of the effect of hexarelin on other anterior pituitary hormones.

Baseline serum concentrations of PRL, cortisol and TSH (at t = 90 minutes) and the respective peak/trough serum concentration attained in the 90 minutes following the intravenous administration of saline, hexarelin or GHRH-(1-29)-NH_2 are shown in Figures 19, 20 and 21. Intravenous administration of hexarelin resulted in a significant rise in serum PRL and cortisol concentrations (Wilcoxon p = 0.03) but not TSH (Wilcoxon p = 0.35). GHRH-(1-29)-NH_2 resulted in a significant rise in serum PRL and TSH concentrations but not cortisol (Wilcoxon p = 0.03, 0.04 and 0.25, respectively). These data formed the basis for further studies of the PRL- and cortisol- releasing activity of hexarelin (dose-response studies).

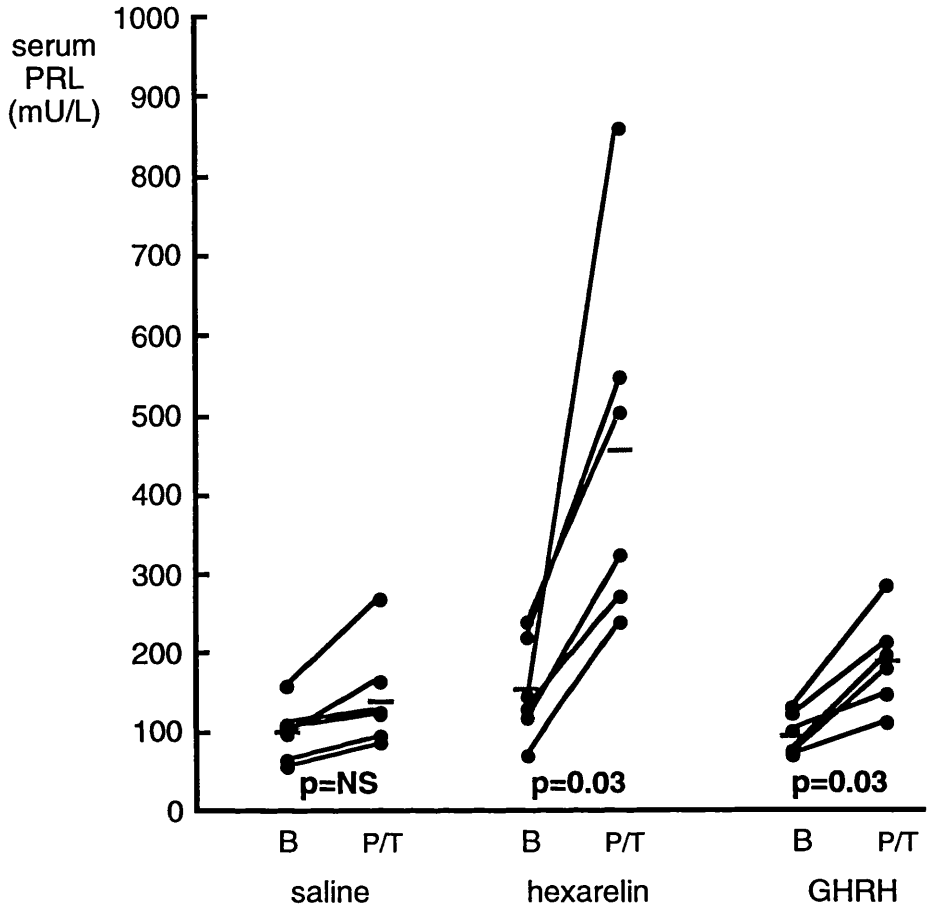


Figure 19. Basal (B) serum PRL concentrations and peak/trough (P/T) concentrations attained following bolus intravenous administration of saline, hexarelin (1.0μg/kg) or GHRH-(1-29)-NH_2 (1.0μg/kg). Horizontal bars indicate mean values.

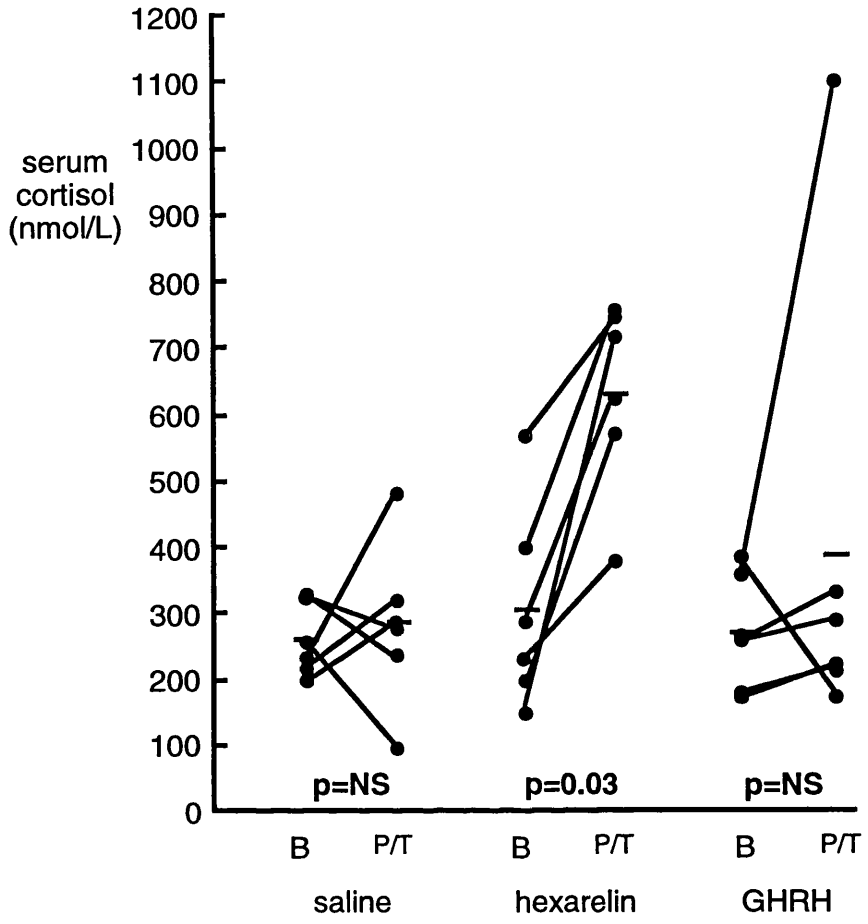


Figure 20. Basal (B) serum cortisol concentrations and peak/trough (P/T) concentrations attained following bolus intravenous administration of saline, hexarelin (1.0μg/kg) or GHRH-(1-29)-NH_2 (1.0 μg/kg). Horizontal bars indicate mean values.

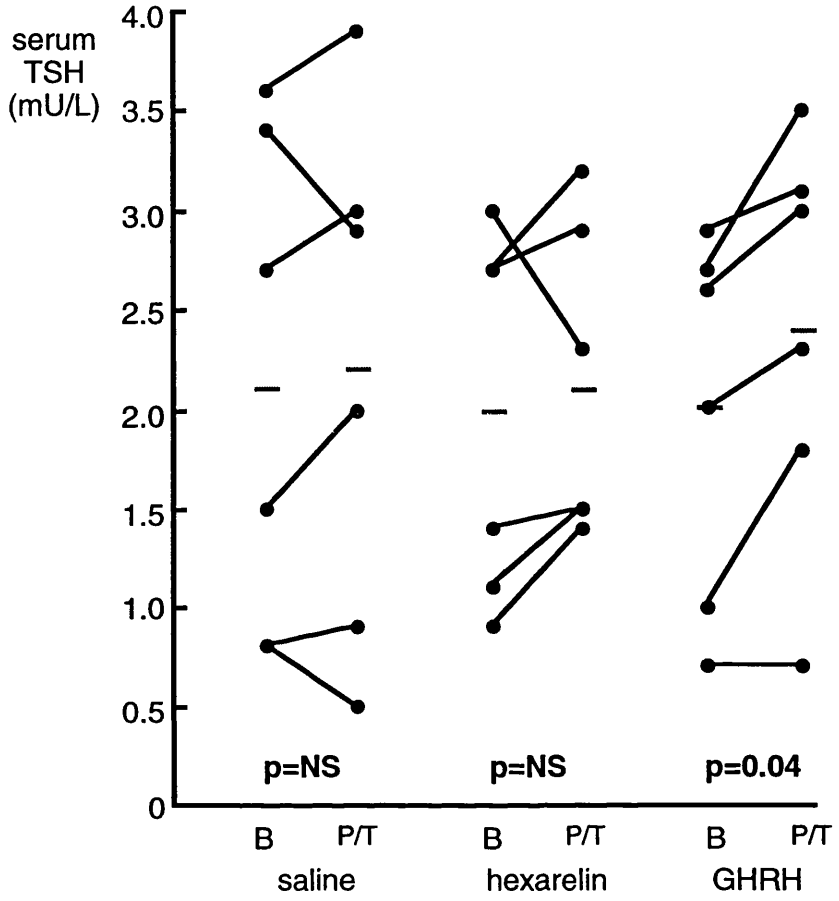


Figure 21. Basal (B) serum TSH concentrations and peak/trough (P/T) concentrations attained following bolus intravenous administration of saline, hexarelin (1.0μg/kg) or GHRH-(1-29)-NH_2 (1.0μg/kg). Horizontal bars indicate mean values.

Summary

1. The prior administration of exogenous r-hGH attenuated significantly the GH-response to hexarelin or GHRH-(1-29)-NH_2.

2. The GH response to hexarelin was greater than that to GHRH-(1-29)-NH_2, irrespective of whether administration of the secretagogue was preceded by r-hGH or saline administration.

3. The intravenous administration of 100 mU of r-hGH resulted in physiological serum GH concentrations, but did not alter serum IGF-1 levels during the study sessions.

4. The intravenous administration of hexarelin caused significant elevation of serum PRL and cortisol, but not TSH, concentrations. This called for further studies, the results of which are reported under "Dose-response studies of the GH, PRL and cortisol response to hexarelin".

Section 3. The Effects of Somatostatin Tone and Somatostatin Withdrawal on the Growth Hormone Response to Hexarelin, Growth Hormone Releasing Hormone and Hexarelin plus Growth Hormone Releasing Hormone

Studies of somatostatin dose

a) Serum growth hormone concentration profile

Figure 22 shows the serum GH concentration profiles obtained following the intravenous bolus administration of hexarelin during a constant intravenous infusion of saline, SS(1-14) 20 $\mu g/m^2/hr$ (SS20) or SS(1-14) 50 $\mu g/m^2/hr$ (SS50) (protocol design shown in Figure 8). Serum GH concentrations were attenuated during SS20 infusion compared to saline, with further attenuation during SS50 infusion.

b) Peak serum growth hormone responses

The intravenous infusion of SS20 resulted in a significant reduction in peak serum GH concentration following bolus intravenous administration of hexarelin, GHRH-(1-29)-NH_2 or hexarelin plus GHRH-(1-29)-NH_2 at t = 0 minutes, as shown in Figure 23. Infusing a higher dose of SS(1-14), namely 50 $\mu g/m^2/hr$ (SS50), resulted in a further reduction in the peak serum GH concentrations, though these did not differ significantly from the responses obtained during the SS20 infusion (Figure 23).

The maximum serum GH concentration in the 30 minute period leading up to the intravenous administration of saline or GH secretagogue(s) at t = 0 minutes was similar in all study groups (one-way ANOVA $p > 0.05$; data shown in Table 13). This indicates that the above findings

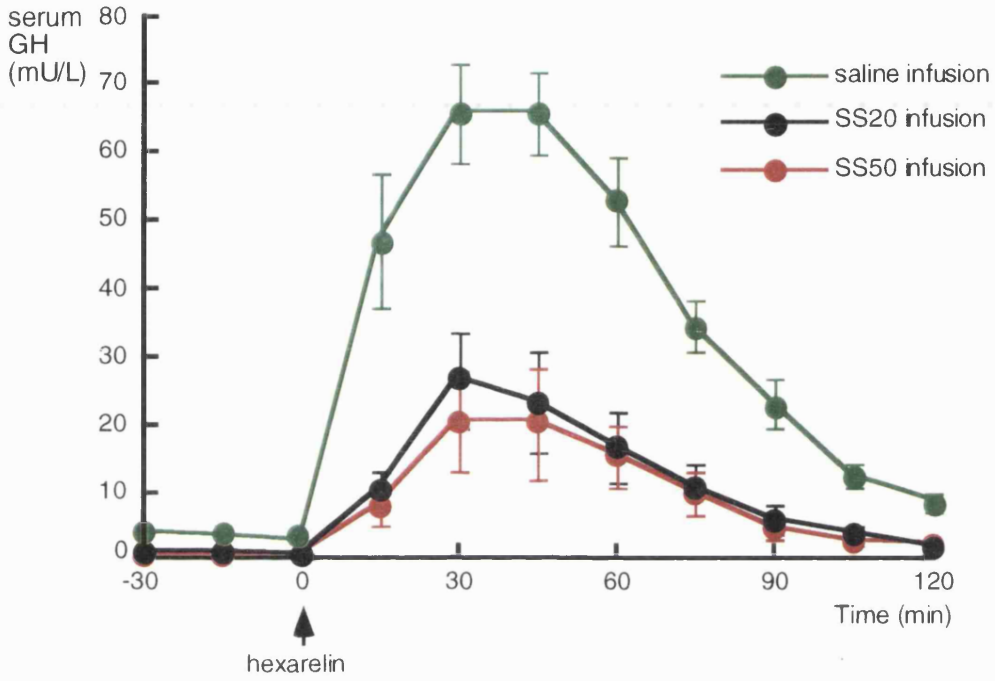


Figure 22. Serum GH concentration profiles obtained during a constant intravenous infusion of saline, SS(1-14) (20mg/m^2/hr) or SS(1-14) (50mg/m^2/hr) with an intravenous bolus of hexarelin (1.0mg/kg) being administered 60 minutes (t = 0 min) after the commencement of the infusion (Figure 8, studies 2, 6 and 10). Data shown as mean ± SEM.

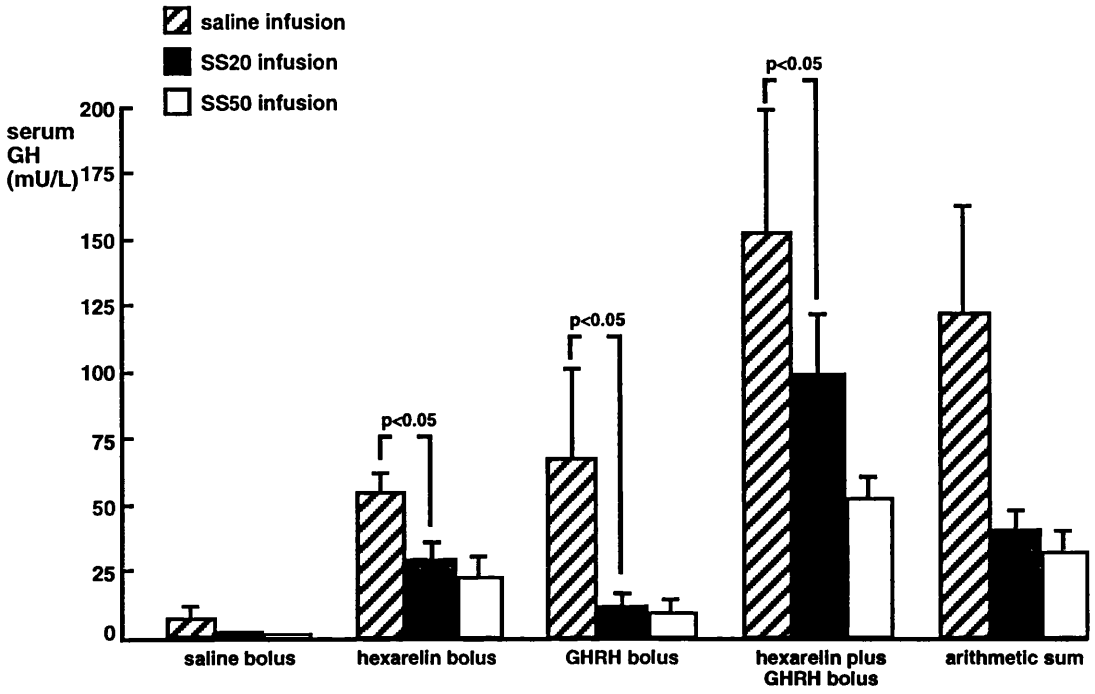


Figure 23. Peak serum GH concentrations following the intravenous administration of saline, hexarelin (1.0μg/kg), GHRH-(1-29)-NH_2 (1.0μg/kg) or hexarelin (1.0μg/kg) plus GHRH-(1-29)-NH_2 (1.0μg/kg) at t = 0 min during a constant intravenous infusion of saline, SS(1-14) (20μg/m^2/hr) or SS(1-14) (50μg/m^2/hr) from t = -60 to t = 120 min. The arithmetic sum of the peak serum GH concentrations following the isolated administration of hexarelin (1.0μg/kg) or GHRH-(1-29)-NH_2 (1.0μg/kg) are shown for comparison with those following the coadministration of the two secretagogues. Data shown as mean + SEM.

were not due to differences in the serum GH status at the time of bolus administration of secretagogue(s).

The peak serum GH concentrations following the combined administration of hexarelin plus GHRH-(1-29)-NH_2 were greater than the sum of the respective peak serum GH concentrations following their isolated administration, irrespective of whether saline, SS20 or SS50 was being infused, as shown in Figure 23. However, the difference in peak serum GH concentrations did not reach statistical significance (Wilcoxon $p > 0.05$).

Data for Figures 22 and 23 and Table 13 are given in Appendix 5.

Table 13. Maximum serum GH concentrations in the 30 minute period leading up to the time of administration of hexarelin, GHRH-(1-29)-NH_2 or both together whilst a constant rate intravenous infusion of saline, SS (20 $\mu g/m^2/hr$) or SS (50 $\mu g/m^2/hr$) was being given. Data shown as mean ± SEM.

Infusion	serum GH concentration pre hexarelin bolus	serum GH concentration pre GHRH-(1-29)-NH_2 bolus	serum GH concentration pre hexarelin plus GHRH-(1-29)-NH_2 bolus
saline	2.3 ± 0.8 mU/L	4.6 ± 3.5 mU/L	1.0 ± 0.3 mU/L
SS20	1.3 ± 0.5 mU/L	1.9 ± 0.7 mU/L	0.7 ± 0.2 mU/L
SS50	0.8 ± 0.1 mU/L	2.6 ± 1.6 mU/L	0.8 ± 0.1 mU/L

Studies of somatostatin withdrawal

a) Serum growth hormone concentration profile

Figure 24 shows the serum GH concentration profiles obtained during and following the withdrawal of the constant intravenous infusion of saline or SS20 and the intravenous bolus

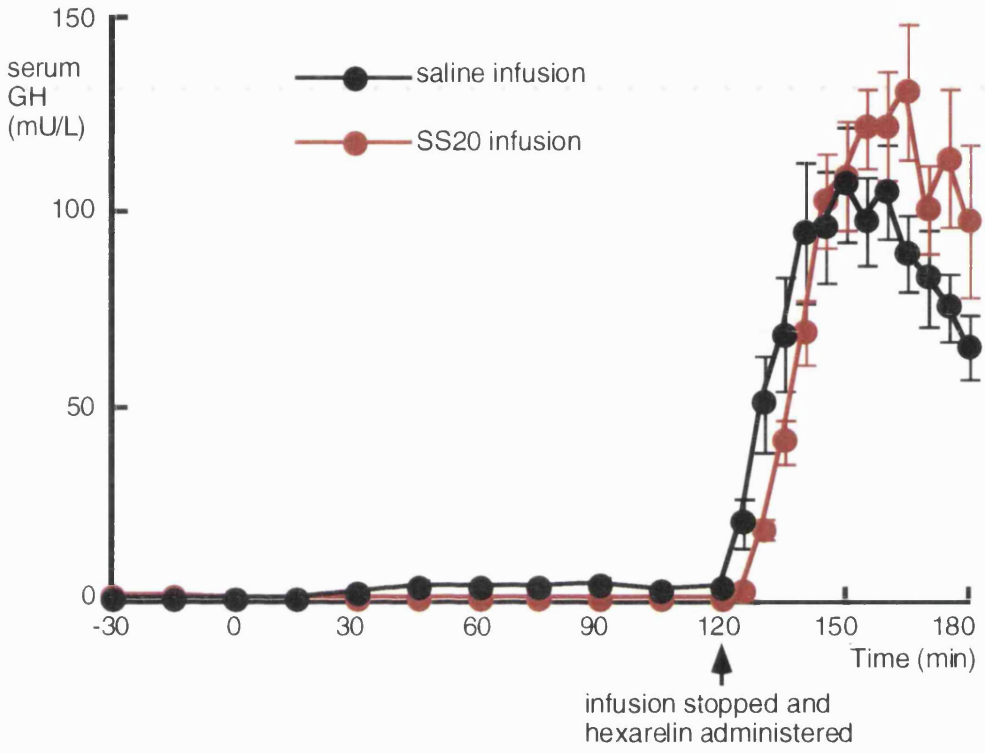


Figure 24. Serum GH concentration profiles obtained during and following the withdrawal of a constant intravenous infusion of saline or SS(1-14) (20mg/m²/hr) with an intravenous bolus of hexarelin (1.0mg/kg) being administered at the time of infusion withdrawal (Figure 9a, studies 2 and 6). Data shown as mean ± SEM.

administration of hexarelin at the time of infusion withdrawal (Figure 9a, studies 2 and 6). Withdrawal of SS20, as opposed to saline, resulted in a greater GH response to bolus intravenous administration of hexarelin.

b) Peak serum growth hormone responses

Withdrawal of SS20 infusion at the time of bolus intravenous hexarelin administration resulted in a slight but significant increase in peak serum GH concentration (Wilcoxon $p = 0.03$) (Figure 25). The peak GH response to GHRH-(1-29)-NH_2 and hexarelin plus GHRH-(1-29)-NH_2 was not affected by SS20 withdrawal (Wilcoxon p = NS) (Figure 25).

The time taken to reach peak serum GH concentration following intravenous bolus administration of hexarelin, GHRH-(1-29)-NH_2 or hexarelin plus GHRH-(1-29)-NH_2 and the withdrawal of the SS20 infusion compared to that following the same bolus administration and the withdrawal of saline infusion was not statistically different between any two paired groups (Wilcoxon $p > 0.05$; data shown in Table 14). However, there was a general trend towards a longer time to reach peak serum GH concentration following SS20 withdrawal.

Data for Figures 24 and 25 and Table 14 are given in Appendix 6.

Table 14. Time taken to reach peak serum GH concentration following the withdrawal of a saline or SS (20 $\mu g/m^2/hr$) infusion and the simultaneous intravenous administration of hexarelin, GHRH-(1-29)-NH_2 or both together. Data shown as mean ± SEM. Median values are shown in brackets.

Infusion	Time to peak GH following hexarelin bolus	Time to peak GH following GHRH-(1-29)-NH_2 bolus	Time to peak GH following hexarelin plus GHRH-(1-29)-NH_2 bolus
saline	32.5 ± 3.1 (32.5) min	33.3 ± 7.5 (30) min	32.5 ± 4.4 (35) min
SS20	38.3 ± 2.5 (37.5) min	46.7 ± 2.5 (47.5) min	40.8 ± 5.1 (37.5) min

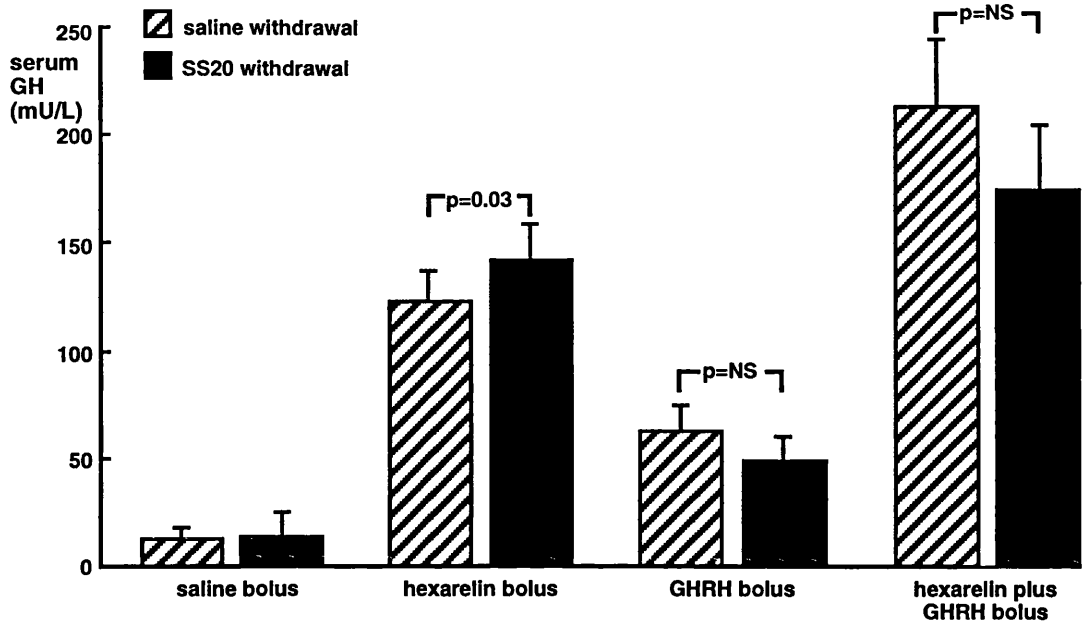


Figure 25. Peak serum GH concentrations following the intravenous administration of saline, hexarelin (1.0μg/kg), GHRH-(1-29)-NH_2 (1.0μg/kg) or hexarelin (1.0μg/kg) plus GHRH-(1-29)-NH_2 (1.0μg/kg) and the simultaneous cessation of a three-hour constant intravenous infusion of either saline or SS(1-14) (20μg/m^2/hr). Data shown as mean + SEM.

c) The effect of pretreatment with hexarelin or GHRH-(1-29)-NH_2 on the growth hormone response to somatostatin withdrawal

The protocol design of these studies is shown in Figure 9b. SS50 infusion was able completely to suppress GH secretion within 60 minutes of the start of the infusion and again by two hours after bolus administration at t = 0 minutes of saline, hexarelin or GHRH-(1-29)-NH_2 (serum GH concentrations at t = 0 and t = 120 minutes were all below the lower limit of the detection of the assay). Despite a small GH response to hexarelin and GHRH-(1-29)-NH_2 following their respective bolus administration at t = 0 minutes (hexarelin peak serum GH concentration: 6.8 ± 3.6 mU/L, GHRH-(1-29)-NH_2 peak serum GH concentration: 2.4 ± 0.53 mU/L), withdrawal of SS50 infusion did not result in a rise in serum GH concentrations in any of the studies.

Summary

1. SS(1-14) attenuated the GH response to hexarelin, GHRH-(1-29)-NH_2 and hexarelin plus GHRH-(1-29)-NH_2. Although only two doses of SS(1-14) were tested, the degree of attenuation appeared to be dose-related.

2. The GH response to hexarelin was not abolished even in the presence of high dose (50 $\mu g/m^2/hr$) of SS(1-14).

3. Under the same conditions of SS(1-14) inhibition, hexarelin was able to produce a greater GH response compared with GHRH-(1-29)-NH_2.

4. Hexarelin plus GHRH-(1-29)-NH_2 had potent GH-releasing activity despite a high SS(1-14) dose of 50 $\mu g/m^2/hr$.

5. Under each of the three experimental conditions used (saline, low and high dose SS(1-14) infusions), the peak GH responses following the combined administration of hexarelin plus

GHRH-(1-29)-NH_2 were greater than the sum of the peak GH responses following their isolated administration.

6. Withdrawal of SS(1-14) resulted in a slight increase in the GH response to hexarelin but not that to GHRH-(1-29)-NH_2, alone or in combination with hexarelin.

7. The time taken to reach peak serum GH concentration following bolus intravenous administration of secretagogue(s) was generally longer following SS20 withdrawal compared to saline withdrawal. However, this difference was not statistically significant.

8. Pretreatment with either hexarelin or GHRH-(1-29)-NH_2 during SS(1-14) (50 $\mu g/m^2/hr$) infusion did not influence GH release following (SS-14) withdrawal.

Section 4. Dose-Response Studies of the Growth Hormone, Prolactin and Cortisol Response to Hexarelin

Dose-response curves

a) Growth hormone dose-response curve

cubic model

Using the peak serum GH concentrations for each subject following the administration of intravenous saline and varying doses of hexarelin, and applying the curve fitting function of the statistics package SPSS, a curve was constructed and is shown in Figure 26. The best fit GH dose-response curve for *all* subjects was described by the cubic equation:

$y = 0.39 + 47.4\ (\pm 159.1)\ (\text{hexarelin dose}) + 264.9\ (\pm 403.8)\ (\text{hexarelin dose})^2 - 182\ (\pm 269.5)\ (\text{hexarelin dose})^3$,

where y = serum GH concentration, and values in brackets represent ± SEM.

The curve reached a plateau at a serum GH concentration of approximately 140 mU/L corresponding to a hexarelin dose of 1.0 μg/kg. The ED50, obtained by curve fitting *each* subject's peak serum GH concentration following intravenous saline or varying doses of hexarelin, plotted against the dose administered, was 0.48 ± 0.02 μg/kg (mean ± SEM).

It can be seen from Figure 26 that a hexarelin dose as low as 0.2 μg/kg would be expected to generate a peak GH response of 20 mU/L.

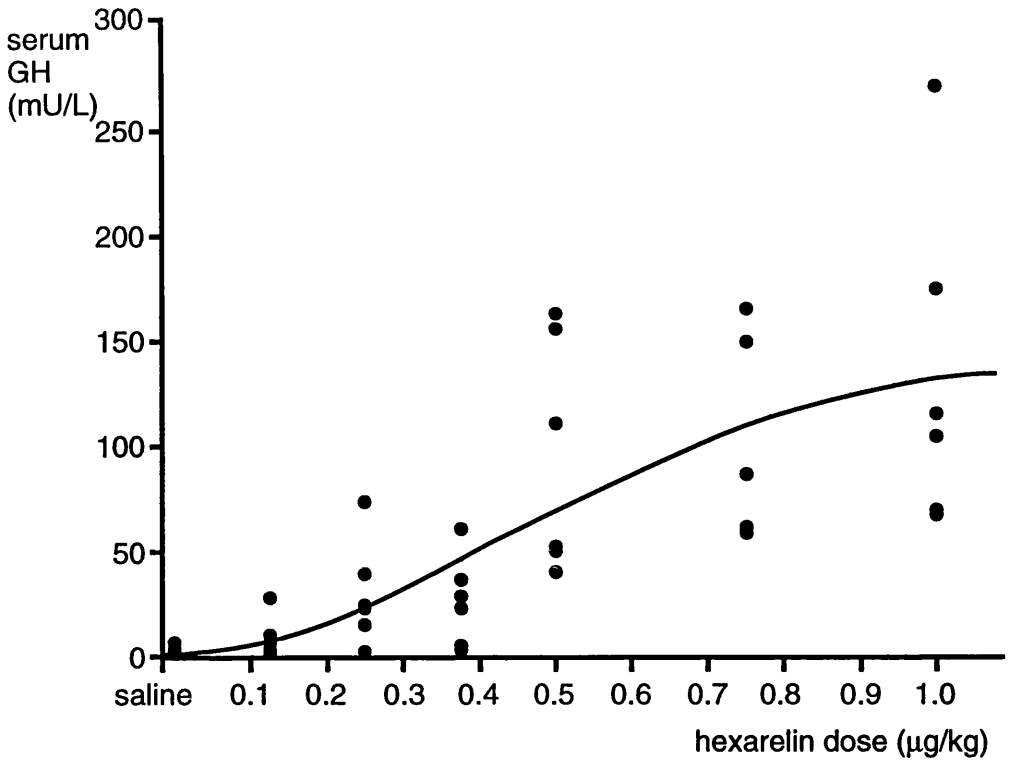


Figure 26. Dose-response curve of intravenous hexarelin dose plotted on the *x-axis vs* peak serum GH concentration plotted on the *y-axis*. The S-shaped curve reached a plateau at a GH concentration of 140 mU/L.

linear model

A plot of the $\log_{10}$ hexarelin doses against the peak GH response is shown in Figure 27. Hexarelin doses were expressed in nanograms/kg before they were $\log_{10}$ transformed in order to avoid the use of negative figures. The relationship between the two variables was described by the linear equation:

$y = 140.5 (\pm 26.1) (\log_{10}$ hexarelin dose) - 301.9,

where y = serum GH concentration, and the value in brackets represents ± SEM.

Subject effect on the peak GH response to hexarelin

Figure 28 shows a dot plot of the peak GH responses against the $\log_{10}$ hexarelin dose, showing the rank variability of each subject's response to the various hexarelin doses administered. Hexarelin doses were expressed in nanograms/kg before they were $\log_{10}$ transformed in order to avoid the use of negative figures. Two-way simple factorial ANOVA showed a significant dose effect ($p < 0.0005$) as well as a significant subject effect ($p = 0.009$).

Each subject's peak GH response to each hexarelin dose was ranked from 1 (lowest peak amongst all subjects for a particular dose) to 6 (highest peak). The data are shown in Table 15.

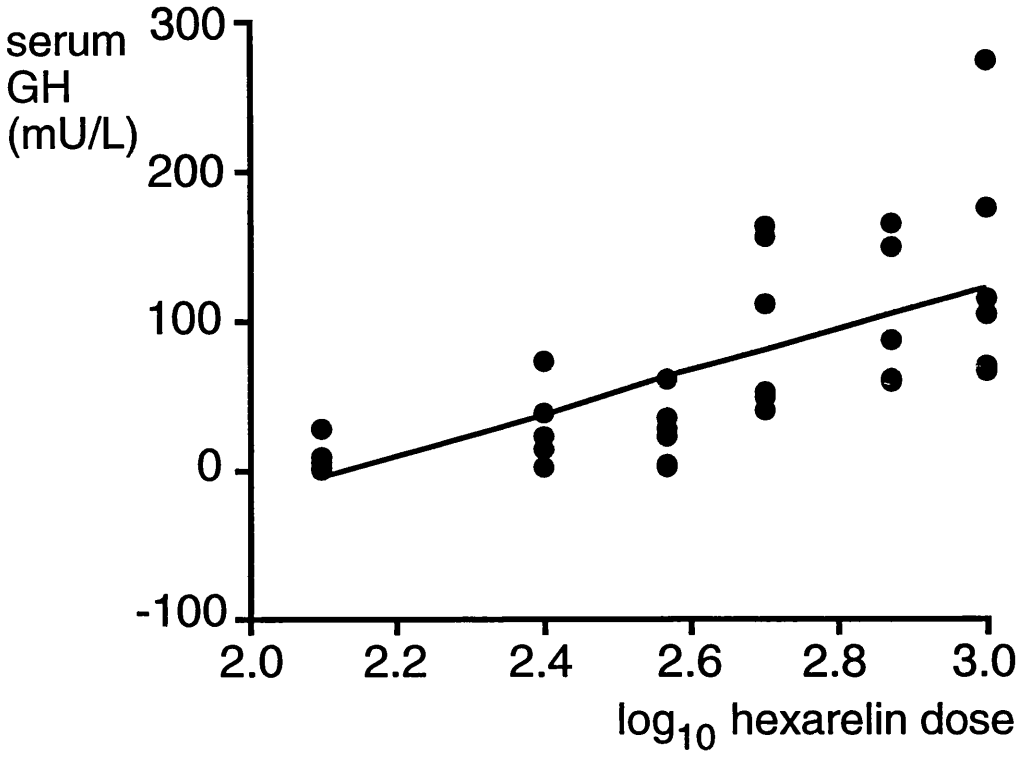


Figure 27. Dose-response curve of $\log_{10}$ intravenous hexarelin dose plotted on the *x-axis vs* peak serum GH concentration plotted on the *y-axis*, showing the linear relationship. Hexarelin doses were expressed in nanograms/kg before they were $\log_{10}$ transformed in order to avoid the use of negative figures.

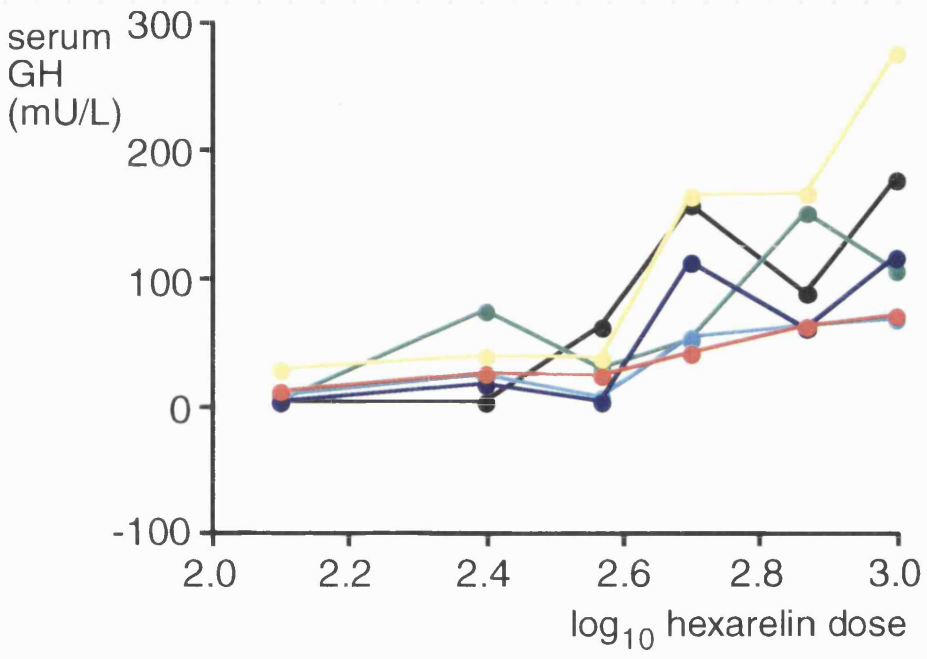


Figure 28. Dot plot of the peak serum GH concentration *vs* the $\log_{10}$ intravenous hexarelin dose, showing the rank variability of each subject's response to the various hexarelin doses administered. Hexarelin doses were expressed in nanograms/kg before they were $\log_{10}$ transformed in order to avoid the use of negative figures. The response of each subject is shown in a different colour.

Table 15. Rank of each subject's peak GH response to each hexarelin dose (1 = lowest peak amongst all subjects; 6 = highest peak).

Subject	hexarelin 0.125 μg/kg	hexarelin 0.25 μg/kg	hexarelin 0.375 μg/kg	hexarelin 0.50 μg/kg	hexarelin 0.75 μg/kg	hexarelin 1.0 μg/kg
RH	5	4	3	1	3	2
AD	1	2	1	4	1	4
RR	6	5	5	6	6	6
MH	4	3	2	3	2	1
DS	3	6	4	2	5	3
JA	2	1	6	5	4	5

b) Prolactin dose-response curve

cubic model

The PRL dose-response curve, constructed using the maximum change in serum PRL concentration following the administration of saline or varying doses of hexarelin compared with baseline values measured at t = 0 minutes, is shown in Figure 29. The best fit curve for the maximum change in PRL concentration for *all* subjects was described by the cubic equation:

$y = 50.57 - 227.5\ (\pm 413.8)\ (\text{hexarelin dose}) + 1560.4\ (\pm 1049.8)\ (\text{hexarelin dose})^2 - 1136.8\ (\pm 700.7)\ (\text{hexarelin dose})^3$,

where y = maximum change in serum PRL concentration, and values in brackets represent ± SEM.

The curve reached a plateau at approximately 300 mU/L (maximum change). The maximum rise in PRL concentration from baseline values at t = 0 minutes occurred between hexarelin doses of 0.75 μg/kg to 1.0 μg/kg. The ED50, obtained by curve fitting *each* subject's data, as described above, was 0.40 ± 0.05 μg/kg (mean ± SEM).

It can be seen from Figure 29 that at low doses of hexarelin, such as 0.2 μg/kg, the rise in PRL concentration is modest (approximately 60 mU/L).

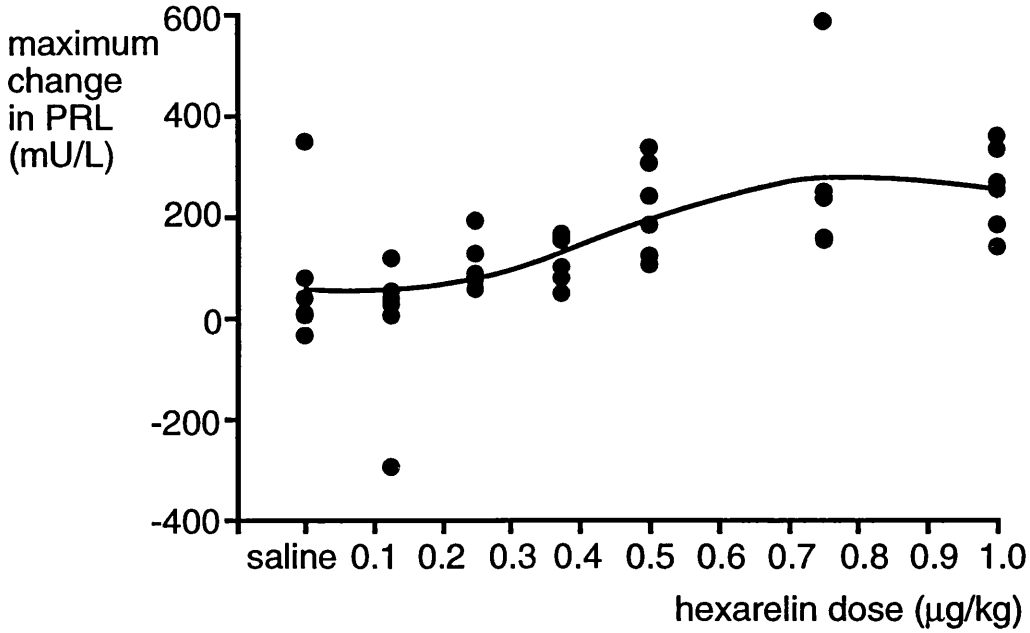


Figure 29. Dose-response curve of intravenous hexarelin plotted on the *x-axis vs* maximum change in serum prolactin concentration from baseline value at t = 0 min plotted on the *y-axis*. The S-shaped curve reached a plateau at a maximum change of approximately 300 mU/L.

linear model

A plot of the $\log_{10}$ hexarelin doses against the maximum change in serum PRL concentration is shown in Figure 30. Hexarelin doses were expressed in nanograms/kg before they were $\log_{10}$ transformed in order to avoid the use of negative figures. The relationship between the two variables was described by the linear equation:

$y = 310.2 (\pm 57.6) (\log_{10}$ hexarelin dose) - 654

where y = maximum change in serum PRL concentration, and the value in brackets represents ± SEM.

Subject effect on the maximum change in serum prolactin concentration following hexarelin administration

Figure 31 shows a dot plot of the maximum change in serum PRL concentration against the $\log_{10}$ hexarelin dose, showing the rank variability of each subject's response to the various hexarelin doses administered. Hexarelin doses were expressed in nanograms/kg before they were $\log_{10}$ transformed in order to avoid the use of negative figures. Two-way simple factorial ANOVA showed a significant dose effect ($p = 0.001$), but no significant subject effect ($p = 0.161$).

Each subject's maximum change in serum PRL concentration to each hexarelin dose was ranked from 1 (least change amongst all subjects for a particular dose) to 6 (greatest change). The data are shown in Table 16.

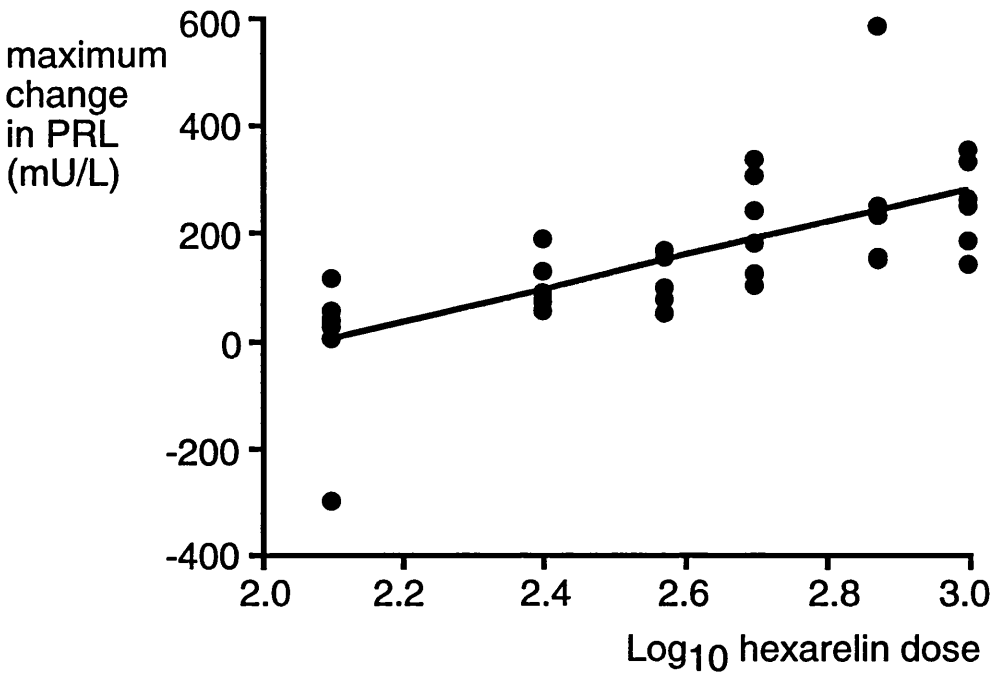


Figure 30. Dose-response curve of $\log_{10}$ intravenous hexarelin dose plotted on the *x-axis vs* maximum change in serum PRL concentration from baseline value at t = 0 min plotted on the *y-axis*, showing the linear relationship. Hexarelin doses were expressed in nanograms/kg before they were $\log_{10}$ transformed in order to avoid the use of negative figures.

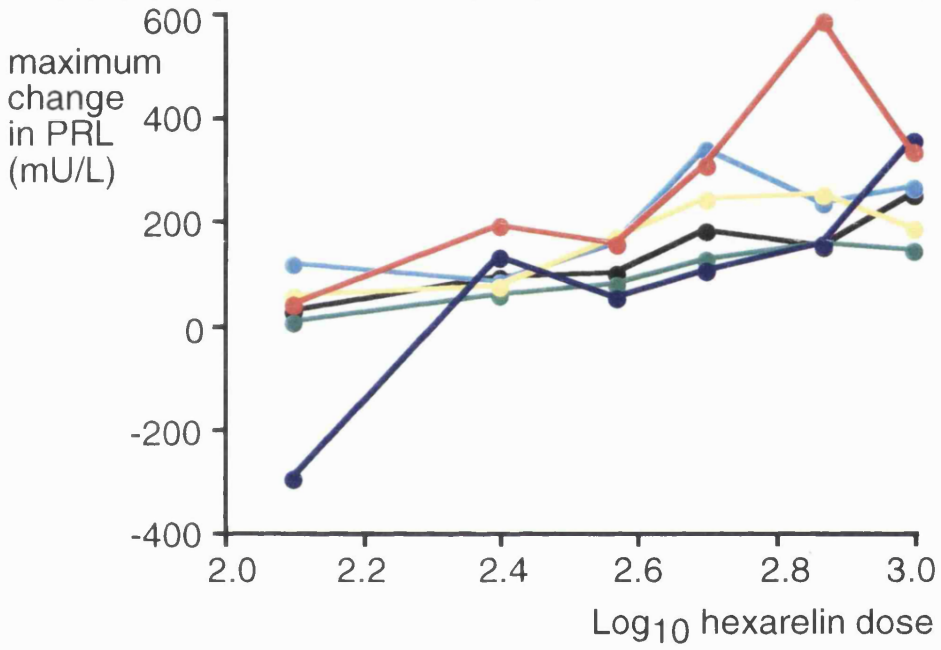


Figure 31. Dot plot of the maximum change in serum PRL concentration *vs* the $\log_{10}$ intravenous hexarelin dose, showing the rank variability of each subject's response to the various hexarelin doses administered. Hexarelin doses were expressed in nanograms/kg before they were $\log_{10}$ transformed in order to avoid the use of negative figures. The response of each subject is shown in a different colour.

Table 16. Rank of each subject's maximum change in serum PRL concentration to each hexarelin dose (1 = least change; 6 = greatest change).

Subject	hexarelin 0.125 μg/kg	hexarelin 0.25 μg/kg	hexarelin 0.375 μg/kg	hexarelin 0.50 μg/kg	hexarelin 0.75 μg/kg	hexarelin 1.0 μg/kg
RH	4	6	5	5	6	5
AD	1	5	1	1	3	6
RR	5	2	6	4	5	2
MH	6	3	4	6	4	4
DS	2	1	2	2	2	1
JA	3	4	3	3	1	3

c) Cortisol dose-response curve

The dot-plot of increasing doses of intravenous hexarelin versus the maximum change in serum cortisol concentration compared to baseline values at t = 0 minutes (Figure 32) showed no clear dose-response effect. The maximum changes in serum cortisol concentration following the intravenous administration of low doses of hexarelin (≤ 0.375 μg/kg) were equally distributed around the zero line, with no clear-cut rise or fall in concentration, although the median values were slightly positive. However, at higher doses (≥ 0.5 μg/kg) the change was predominantly an increase. The data could be described as a step change to a new plateau of maximum change in serum cortisol concentration. The median values for the maximum change in serum cortisol concentrations are shown in Table 17.

Table 17. Median values for the maximum change in serum cortisol concentration following the administration of varying doses of hexarelin. A step change is observed between the doses 0.375 μg/kg and 0.50 μg/kg.

hexarelin dose	Maximum change in serum cortisol concentration (median values)
0.0 (saline)	-18.5 nmol/L
0.125 μg/kg	7.0 nmol/L
0.25 μg/kg	39.5 nmol/L
0.375 μg/kg	35.5 nmol/L
0.50 μg/kg	97.5 nmol/L
0.75 μg/kg	82.5 nmol/L
1.0 μg/kg	95.0 nmol/L

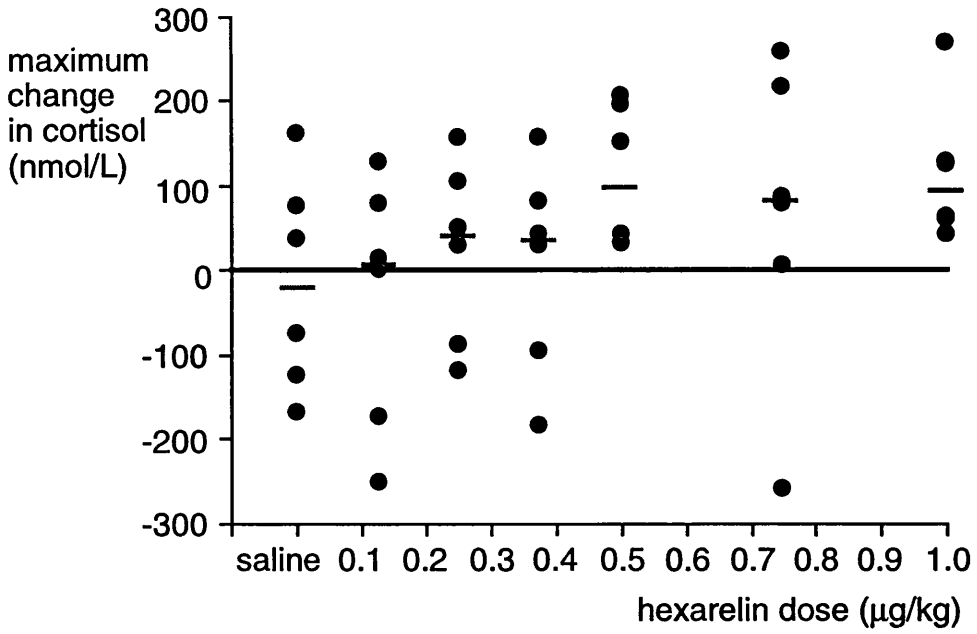


Figure 32. Dose-response of intravenous hexarelin plotted on the *x-axis vs* maximum change in serum cortisol concentration from baseline value at t = 0 min plotted on the *y-axis*. The dot plot shows a step to a new plateau of approximately 90 nmol/L increase in cortisol concentration at a hexarelin dose of 0.5µg/kg. Median values are represented by horizontal bars.

linear model

A plot of the $\log_{10}$ hexarelin doses against the maximum change in serum cortisol concentration is shown in Figure 33. Hexarelin doses were expressed in nanograms/kg before they were $\log_{10}$ transformed in order to avoid the use of negative figures. The relationship between the two variables was described by the linear equation:

$y = 157.8 (\pm 67.9) (\log_{10}$ hexarelin dose) - 363.9

where y = maximum change in serum cortisol concentration, and the value in brackets represents ± SEM.

Subject effect on the maximum change in serum cortisol concentration following hexarelin administration

Figure 34 shows a dot plot of the maximum change in serum cortisol concentration against the $\log_{10}$ hexarelin dose, showing the rank variability of each subject's response to the various hexarelin doses administered. Hexarelin doses were expressed in nanograms/kg before they were $\log_{10}$ transformed in order to avoid the use of negative figures. Two-way simple factorial ANOVA showed a non-significant dose effect ($p = 0.06$), but a significant subject effect ($p = 0.001$).

Each subject's maximum change in serum cortisol concentration to each hexarelin dose was ranked from 1 (least change amongst all subjects for a particular dose) to 6 (greatest change). The data are shown in Table 18.

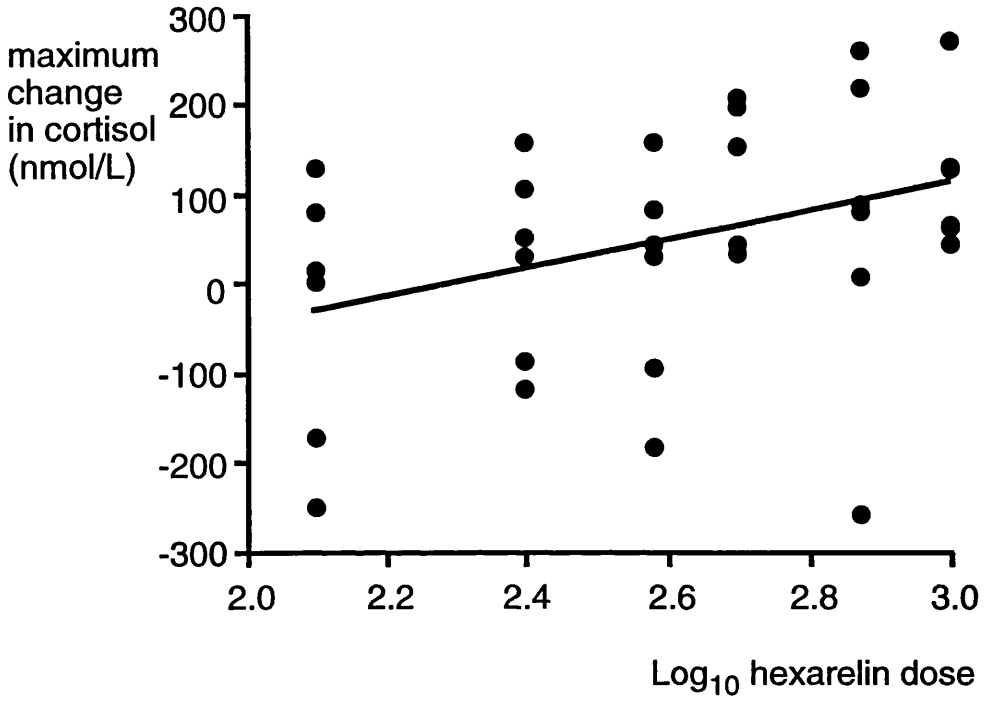


Figure 33. Dose-response curve of $\log_{10}$ intravenous hexarelin dose plotted on the *x-axis vs* maximum change in serum cortisol concentration from baseline value at t = 0 min plotted on the *y-axis*, showing the linear relationship. Hexarelin doses were expressed in nanograms/kg before they were $\log_{10}$ transformed in order to avoid the use of negative figures.

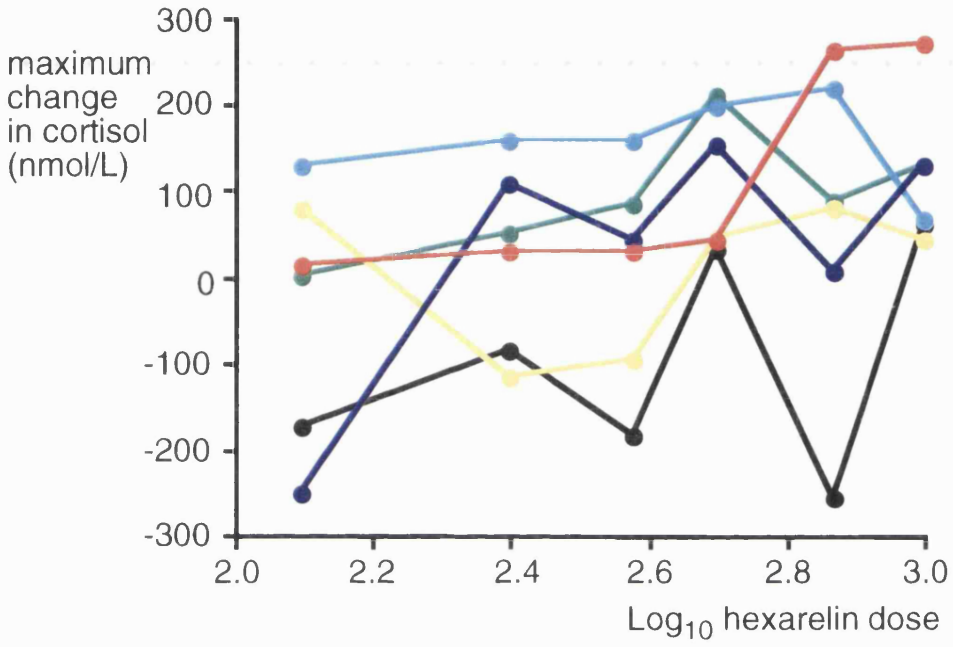


Figure 34. Dot plot of the maximum change in serum cortisol concentration *vs* the $\log_{10}$ intravenous hexarelin dose, showing the rank variability of each subject's response to the various hexarelin doses administered. Hexarelin doses were expressed in nanograms/kg before they were $\log_{10}$ transformed in order to avoid the use of negative figures. The response of each subject is shown in a different colour.

Table 18. Rank of each subject's maximum change in serum cortisol concentration to each hexarelin dose (1 = least change; 6 = greatest change).

Subject	hexarelin 0.125 μg/kg	hexarelin 0.25 μg/kg	hexarelin 0.375 μg/kg	hexarelin 0.50 μg/kg	hexarelin 0.75 μg/kg	hexarelin 1.0 μg/kg
RH	4	3	3	2	6	6
AD	1	5	4	4	2	4
RR	5	1	2	3	4	1
MH	6	6	6	5	5	3
DS	3	4	5	6	3	5
JA	2	2	1	1	1	2

Comparison between the growth hormone- and prolactin-releasing activity of hexarelin

linear model for maximum change in serum growth hormone concentration

A plot of the $\log_{10}$ hexarelin doses against the maximum *change* in serum GH concentration from baseline value at t = 0 minutes is shown in Figure 35. Hexarelin doses were expressed in nanograms/kg before they were $\log_{10}$ transformed in order to avoid the use of negative figures. The relationship between the two variables was described by the linear equation:

$y = 140.4\ (\pm 26.2)\ (\log_{10}\ \text{hexarelin dose}) - 302.8$

where y = maximum change in serum GH concentration, and the value in brackets represents ± SEM.

Using the linear models which described the maximum change in serum GH and PRL concentrations in response to hexarelin, a graph was plotted (Figure 36) which showed that the straight line for the maximum change in PRL concentration was steeper than that for the maximum change in GH concentration. Expressing the data in molar terms showed the same (Figure 37), though the difference in gradient was less marked.

Data for the Figures 26 to 36 and Tables 15 to 18 are given in Appendix 7.

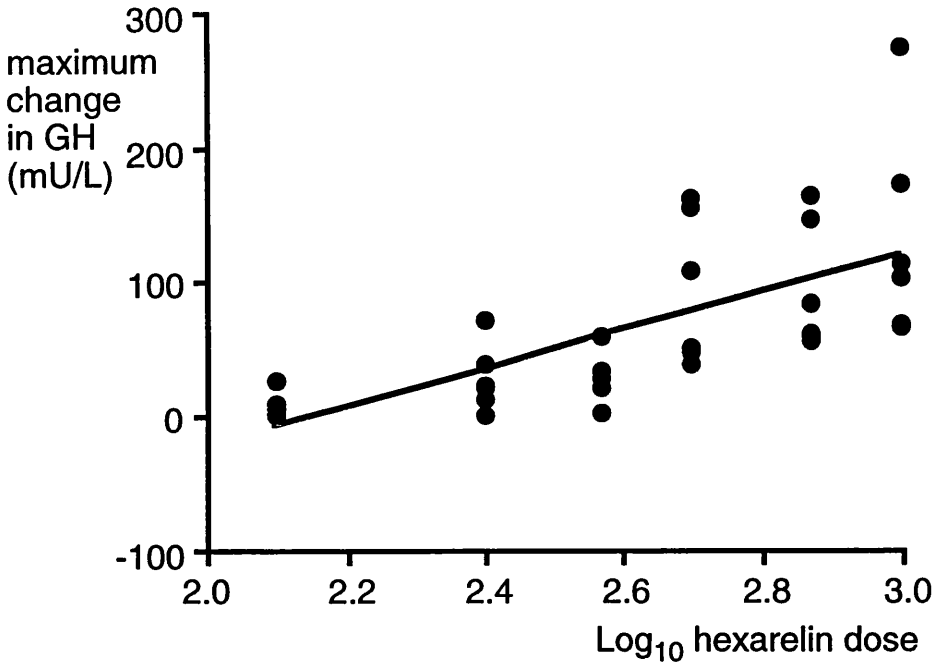


Figure 35. Dose-response curve of $\log_{10}$ intravenous hexarelin dose plotted on the *x-axis vs* maximum change in serum GH concentration from baseline value at t = 0 min plotted on the *y-axis*, showing the linear relationship. Hexarelin doses were expressed in nanograms/kg before they were $\log_{10}$ transformed in order to avoid the use of negative figures.

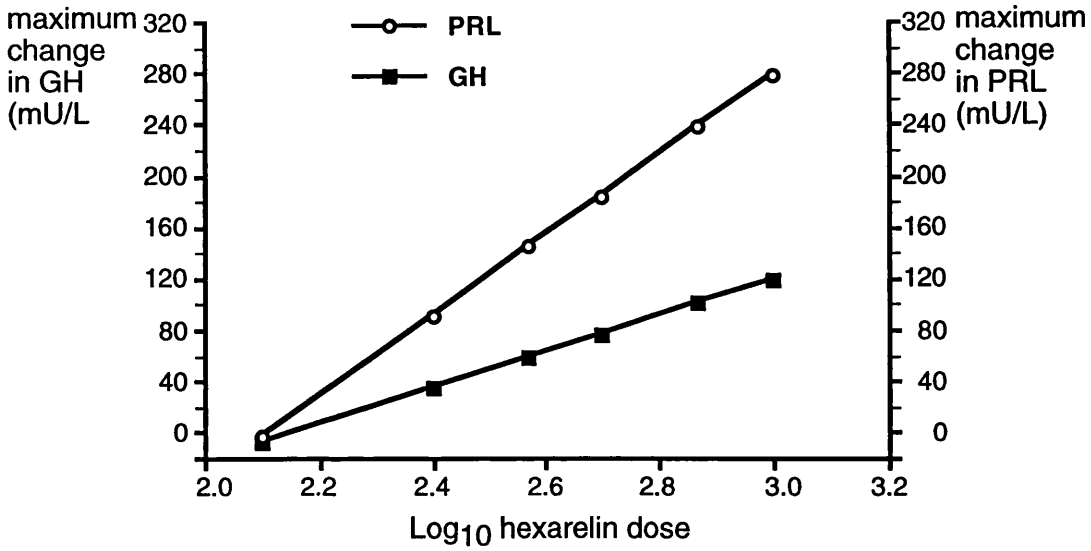


Figure 36. Dose-response curve of $\log_{10}$ intravenous hexarelin dose *vs* maximum change in serum GH and PRL concentrations from baseline values at t = 0 min plotted on the same graph to show the relative activity of hexarelin on the somatotroph and lactotroph. Hexarelin doses were expressed in nanograms/kg before they were $\log_{10}$ transformed in order to avoid the use of negative figures.

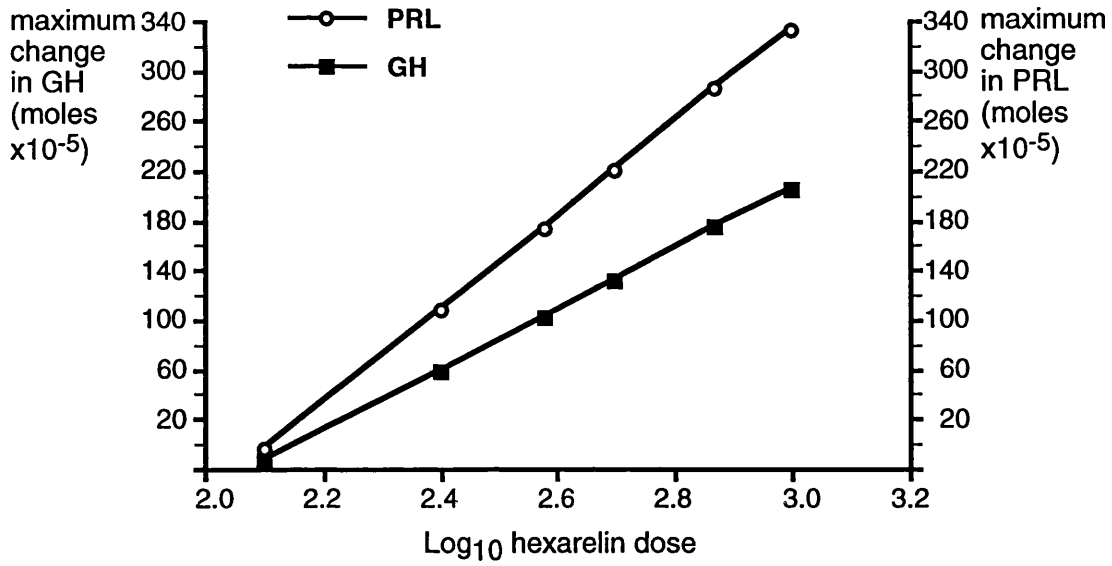


Figure 37. Maximum change from baseline values at t = 0 min in serum GH and PRL in molar terms in response to various doses of hexarelin. Hexarelin doses were expressed in nanograms/kg before they were $\log_{10}$ transformed in order to avoid the use of negative figures.

The effect of hexarelin on serum Thyroid Stimulating Hormone, serum insulin and blood glucose concentrations

The effects of the highest dose of hexarelin (1.0 μg/kg) used (study 7, Figure 10) on serum TSH, serum insulin and blood glucose concentrations are shown in Figures 38, 39 and 40, respectively. For comparison, the effects of intravenous administration of saline (study 1, Figure 10) are shown in the same Figures. Hexarelin (1.0 μg/kg) had no significant effect on serum TSH, serum insulin or blood glucose (two-way ANOVA p = NS).

To address the issue of whether smaller doses of hexarelin had a significant effect on the above parameters, the maximum changes in serum TSH, serum insulin and blood glucose following the administration of saline or varying doses of hexarelin compared with baseline values measured at t = 0 minutes were calculated. These were small in magnitude and failed to show any consistent pattern (Tables 19, 20 and 21, respectively). The maximum changes in serum TSH, serum insulin and blood glucose concentrations were (mean ± SEM) -0.3 ± 0.1 mU/L at a hexarelin dose of 0.125 μg/kg, 1.7 ± 2.7 iU/L at a hexarelin dose of 0.25 μg/kg, and -0.4 ± 0.3 mmol/L at a hexarelin dose of 0.125 μg/kg, respectively.

Data for Figures 38 to 40 and Tables 19 to 21 are given in Appendices 8-10.

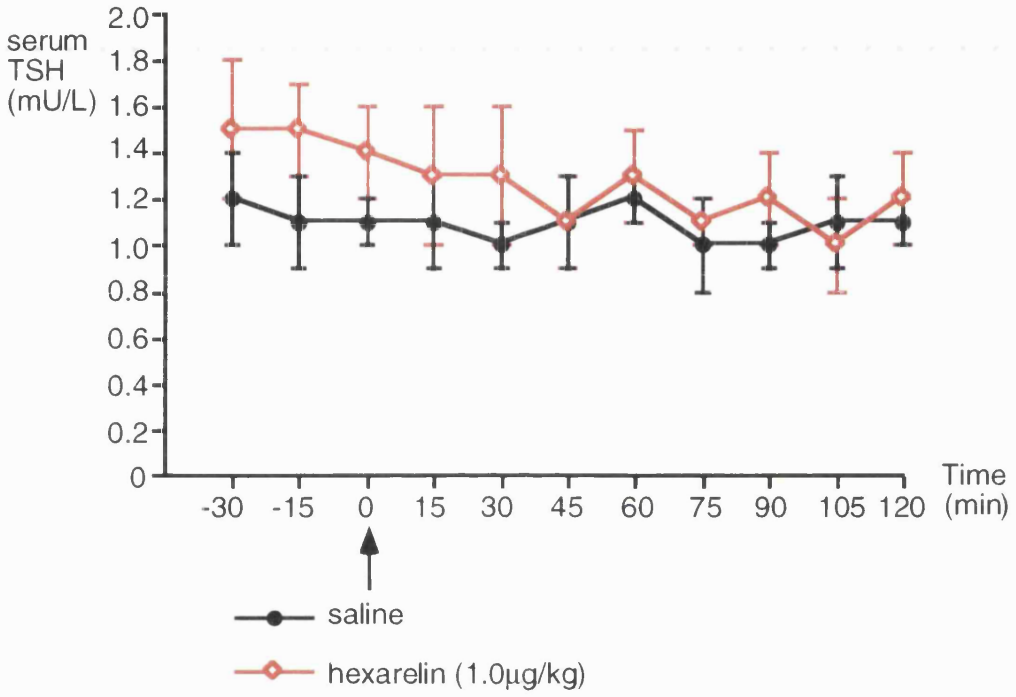


Figure 38. Serum TSH concentration profile in response to an intravenous bolus of hexarelin (1.0µg/kg) or saline. Data shown as mean ± SEM.

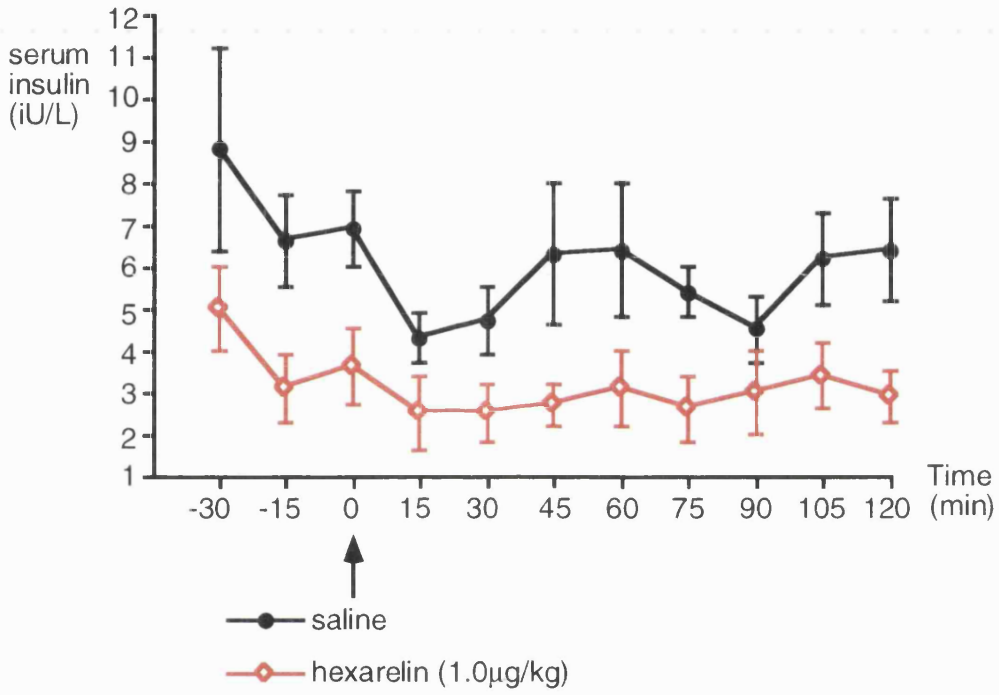


Figure 39. Serum insulin concentration profile in response to an intravenous bolus of hexarelin (1.0µg/kg) or saline. Data shown as mean ± SEM.

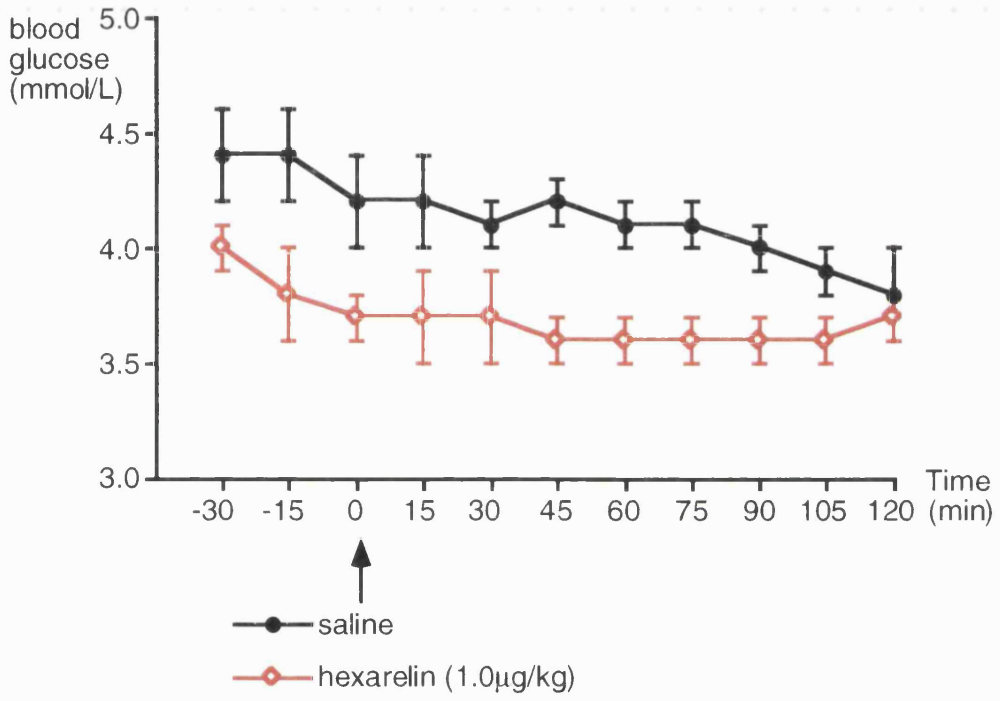


Figure 40. Blood glucose concentration profile in response to an intravenous bolus of hexarelin (1.0µg/kg) or saline. Data shown as mean ± SEM.

Table 19. Maximum change in serum TSH concentration following the administration of saline or varying doses of hexarelin compared with baseline values measured at t = 0 minutes. Data shown as mean ± SEM.

Intravenous bolus	Maximum change in serum TSH concentration compared to baseline values at t = 0 minutes (mU/L)
saline	0.1 ± 0.2
hexarelin (0.125 μg/kg)	- 0.3 ± 0.1
hexarelin (0.25 μg/kg)	0.1 ± 0.1
hexarelin (0.375 μg/kg)	0.1 ± 0.2
hexarelin (0.50 μg/kg)	- 0.2 ± 0.2
hexarelin (0.75 μg/kg)	0.0 ± 0.2
hexarelin (1.0 μg/kg)	0.0 ± 0.3

Table 20. Maximum change in serum insulin concentration following the administration of saline or varying doses of hexarelin compared with baseline values measured at t = 0 minutes. Data shown as mean ± SEM.

Intravenous bolus	Maximum change in serum insulin concentration compared to baseline values at t = 0 minutes (iU/L)
saline	- 2.3 ± 1.6
hexarelin (0.125 μg/kg)	- 0.2 ± 1.5
hexarelin (0.25 μg/kg)	1.7 ± 2.7
hexarelin (0.375 μg/kg)	- 0.5 ± 2.4
hexarelin (0.50 μg/kg)	- 0.2 ± 1.1
hexarelin (0.75 μg/kg)	0.2 ± 0.9
hexarelin (1.0 μg/kg)	- 1.1 ± 1.3

Table 21. Maximum change in blood glucose concentration following the administration of saline or varying doses of hexarelin compared with baseline values measured at t = 0 minutes. Data shown as mean ± SEM.

Intravenous bolus	Maximum change in blood glucose concentration compared to baseline values at t = 0 minutes (mmol/L)
saline	-0.3 ± 0.2
hexarelin (0.125 μg/kg)	- 0.4 ± 0.3
hexarelin (0.25 μg/kg)	0.4 ± 0.4
hexarelin (0.375 μg/kg)	- 0.3 ± 0.3
hexarelin (0.50 μg/kg)	0.0 ± 0.2
hexarelin (0.75 μg/kg)	- 0.2 ± 0.2
hexarelin (1.0 μg/kg)	0.1 ± 0.1

Growth hormone releasing hormone and combined growth hormone releasing hormone plus low dose hexarelin studies

a) Serum growth hormone, prolactin and cortisol data

The peak serum GH responses to GHRH-(1-29)-NH_2 (1.0 μg/kg), hexarelin (0.125 μg/kg), and GHRH-(1-29)-NH_2 (1.0 μg/kg) plus hexarelin (0.125μg/kg) were (mean ± SEM) 42.5 ± 7.8 mU/L, 7.9 ± 4.1 mU/L and 115.9 ± 32.8 mU/L, respectively (Figure 41). The corresponding maximum changes in serum prolactin concentrations were 58.5 ± 17.0, -10.8 ± 59.7 and 88.0 ± 67.1, respectively. There was no rise in serum cortisol concentration following the administration of GHRH-(1-29)-NH_2 (1.0 μg/kg), alone or in combination with low dose hexarelin (0.125 μg/kg), and only a small rise following low dose hexarelin (0.125 μg/kg) alone (median for maximum change from baseline value: 7.0 nmol/L).

b) The effects on serum Thyroid Stimulating Hormone, serum insulin and blood glucose concentrations

The maximum changes in serum TSH, serum insulin and blood glucose concentrations following intravenous bolus administration of GHRH-(1-29)-NH_2 (1.0 μg/kg) were small in magnitude {(mean ± SEM) 0.4 mU/L ± 0.1, -2.4 ± 1.9 iU/L and 0.0 ± 0.2 mmol/L, respectively}, and similarly for those following GHRH-(1-29)-NH_2 (1.0 μg/kg) plus hexarelin (0.125 μg/kg) (-0.1 ± 0.2 mU/L, -2.0 ± 1.3 iU/L, -0.5 ± 0.2 mmol/L, respectively).

GH, PRL, cortisol, TSH, insulin and glucose data for the GHRH and combined GHRH plus low dose hexarelin studies are given in Appendix 11.

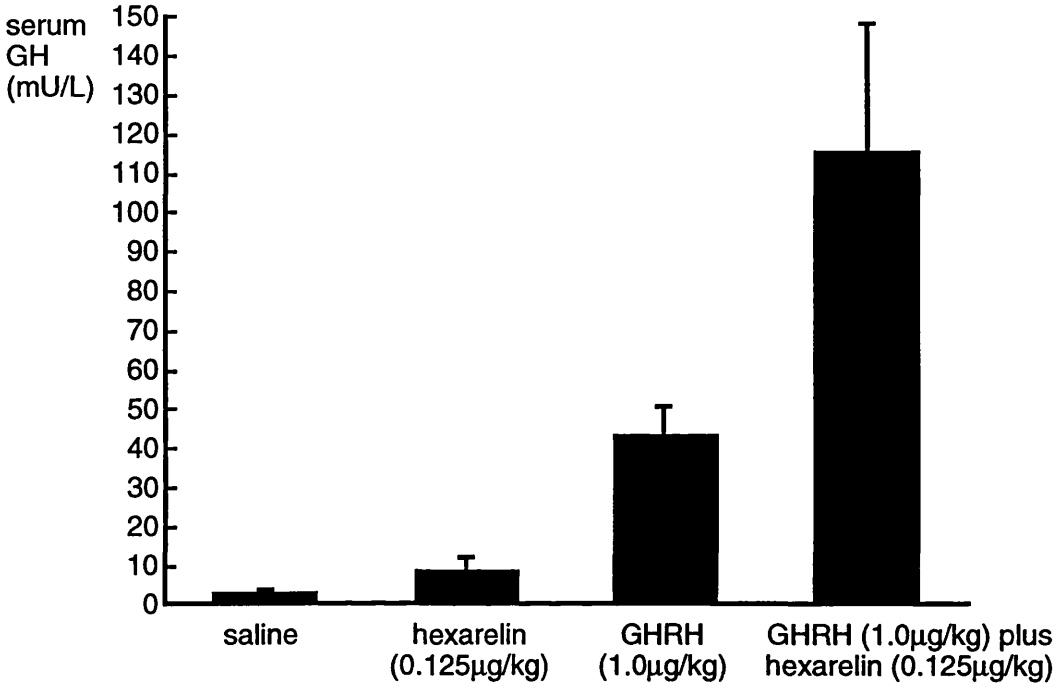


Figure 41. Peak serum GH concentrations following the intravenous bolus administration of saline, hexarelin (0.125µg/kg), GHRH-(1-29)-NH_2 (1.0µg/kg) and GHRH-(1-29)-NH_2 (1.0µg/kg) plus hexarelin (0.125µg/kg). Data shown as mean + SEM.

Summary

1. Hexarelin induced GH release in a dose-dependent manner, with an ED50 of 0.48 μg/kg.

2. Hexarelin was a nonspecific GH secretagogue, inducing the release of PRL in a dose-dependent and cortisol in a stepwise manner.

3. There was a subject effect on the magnitude of the GH and cortisol response to varying doses of hexarelin. No such effect was seen for the PRL response.

4. The PRL-releasing activity of hexarelin was greater than its GH-releasing activity.

5. Hexarelin had no effect on serum TSH, serum insulin or blood glucose concentrations.

6. A low dose of hexarelin (< 0.25 μg/kg) could result in adequate GH release with minimal concomitant release of PRL and cortisol.

7. The combination of low dose hexarelin (0.125 μg/kg) plus GHRH (1.0 μg/kg) resulted in synergistic GH release, moderate elevation of serum PRL concentration but no rise in serum cortisol concentration.

CHAPTER 4

DISCUSSION

Discussion of the results is presented under four main sections. Each section deals with a set of studies (sets 1-4) described in Chapter 2 and results reported in Chapter 3.

Section 1. The Effect of Single and Repeated Doses of Hexarelin, Growth Hormone Releasing Hormone and Hexarelin plus Growth Hormone Releasing Hormone on Growth Hormone Secretion

Pattern and magnitude of the growth hormone response to a single bolus of secretagogue(s)

This set of studies confirmed the potent GH secreting properties of intravenous hexarelin. It demonstrated that co-administration of intravenous hexarelin and GHRH-(1-29)-NH_2 results in a synergistic rather than additive effect, a phenomenon that has been described with other GHRPs (Cheng *et al.*, 1989; Bowers *et al.*, 1990; Bowers, 1993a, Pihoker *et al.*, 1995a). The timing characteristics of the GH response to hexarelin were similar to those following its coadministration with GHRH-(1-29)-NH_2, and were in keeping with data by others (Ghigo *et al.*, 1994a). Using the data from all four sets of studies reported in this thesis, it was possible to demonstrate that the GH response to hexarelin had better repeatability and reproducibility than that to GHRH-(1-29)-NH_2, the former having lower intra- and inter-individual variability.

Potency of hexarelin *vs* growth hormone releasing hormone

On a weight for weight basis, hexarelin was more effective a GH secretagogue than GHRH-(1-29)-NH_2, a finding also reported by Ghigo *et al* (1994a). The dose of hexarelin (1.0 μg/kg) used in this set of studies has been shown to produce near-maximal GH release (Imbimbo *et al.*, 1994; Ghigo *et al.*, 1994a), and is in keeping with my own findings reported in this thesis (see dose-response studies). The dose of GHRH-(1-29)-NH_2 at 1.0 μg/kg is supramaximal (Spoudeas *et al.*, 1994), and was chosen because it is the dose used conventionally when testing the readily releasable pool of GH from the pituitary in humans.

Had a supramaximal dose of hexarelin (> 2.0 μg/kg) or a near-maximal dose of GHRH-(1-29)-NH_2 (0.1 μg/kg) (Spoudeas *et al.*, 1994) been used, the difference in peak serum GH concentrations following these two secretagogues might have been more marked.

Ideally, comparison between hexarelin and GHRH (in terms of GH-releasing potency) should be assessed using equimolar doses (approximately 0.25 μg/kg for hexarelin *vs* 1.0 μg/kg for GHRH-(1-29)-NH_2). Such dosage regimen was contemplated at the outset of my studies but was not followed because most GHRH and GHRP studies to date had been conducted using 1.0 μg/kg and my studies were designed to complement available data. In any case, comparison between the GH-releasing effect of equimolar doses of GHRP and GHRH would be strictly essential if both agents acted through the same receptor which they do not, as discussed in Chapter 1 (see Introduction, Section 3). For the purposes of therapeutic regimens such comparison is less vital since the amount of GH release generated by supramaximal doses of GHRH is less than that generated by near-maximal doses of GHRP.

Synergistic action of hexarelin plus growth hormone releasing hormone

The mechanism for the synergistic action of GHRP plus GHRH is unclear. One mechanism might be the release of an, as yet, unknown (U) hypothalamic factor by a direct action of GHRP (Bowers *et al.*, 1991b; Bowers, 1993a). This U factor might be stimulatory for GH release or, alternatively, might block the release of an inhibitory factor. It is postulated that the U factor would interact with GHRH to bring about synergism. This hypothesis is supported by the absence of synergistic action of GHRP plus GHRH *in vitro* (Sartor *et al.*, 1985; Bowers *et al.*, 1991b), indicating the need for an intact hypothalamus (and hypothalamic-pituitary connection) for synergism to occur.

Although SS would seem a likely target for this postulated hypothalamic factor, GHRPs do not influence its release (Yagi *et al.*, 1990; Guillaume *et al.*, 1994) and the search for an alternative target as well as an endogenous U factor continues. However, hexarelin and other GHRPs may well play an important role in inhibiting the pituitary action of SS (Guillaume *et al.*, 1994; Clark *et al.*, 1989) thereby rendering the somatotroph permissive to the action of

Will it function as as a diagnostic test?

GHRH. This hypothesis is supported further by the results of the SS studies (set 3) reported in this thesis, which showed interaction between SS and hexarelin. Alternatively, the synergistic action might be, in part, due to GHRP-induced endogenous GHRH release (Guillaume *et al.*, 1994), which would cause further GH release. However, the use of supramaximal doses of GHRH-(1-29)-NH_2 (Spoudeas *et al.*, 1994) in our studies, and the finding by others that synergism occurred despite maximal doses of GHRP and GHRH (Peñalva *et al.*, 1993a and 1993b), argues against this being the sole mechanism.

Intra- and inter-individual variability in the growth hormone response to hexarelin

The pooled data from a number of studies reported in this thesis have shown that, in comparison to GHRH-(1-29)-NH_2, hexarelin has a lower GH-response variability, both within and between individuals. The poor repeatability and reproducibility of the GH response to GHRH is well documented (Gelato *et al.*, 1984; Ghigo *et al.*, 1996b), and is the main reason behind the limited usefulness of the "GHRH test" as a test of pituitary GH reserve. The wide variability of the GH response to GHRH has been attributed to its dependence on the state of the SS tone prevailing at the time of bolus GHRH administration. The better repeatability and reproducibility of the GH response to hexarelin could be attributed to its ability to release GH despite the presence of high SS tone, as demonstrated in my studies of SS and hexarelin reported in this thesis. It follows that variation in SS tone would be unlikely to have a marked effect on the GH response to hexarelin. This phenomenon is advantageous should a "hexarelin test" be devised as a diagnostic tool.

Growth hormone response to repeated boluses of secretagogue(s)

In my analysis of the data I applied the principle of deconvolution analysis to determine estimates of the GH secretion rates from the measured serum GH concentrations. The application of this technique is pivotal in interpreting data obtained from a study design which involves repeated administration of a secretagogue within a narrow time interval. Analysis of data on the basis of serum GH concentrations alone in such situations fails to differentiate between hormone secretion and elimination, and may result in misleading conclusions.

Indeed, simple inspection of the data indicated that hexarelin, GHRH-(1-29)-NH_2 or hexarelin plus GHRH-(1-29)-NH_2 was capable of inducing GH release after two successive administrations but also that the levels remained elevated, though decreasing, at the time the second bolus was being administered. These findings indicated the need to deconvolute the data.

Capability of the somatotroph to respond to two successive doses of secretagogue(s)

The GH data, analysed in terms of secretion rates, showed that the somatotroph was capable of responding to two successive doses of intravenous hexarelin or GHRH-(1-29)-NH_2, given alone or in combination and administered 60 or 120 minutes apart.

The capability of the pituitary gland to respond to two successive doses of GHRH has been reported previously (Suri *et al.*, 1991). Previous studies have shown that the pituitary does respond to an intravenous bolus of GHRP following a continuous infusion of the peptide; the response was, however, attenuated (Huhn *et al.*, 1993). No data on the effects of repeated administration of hexarelin were available at the time of execution of this set of studies. Subsequent publications showed, in agreement with my findings, that the somatotroph was capable of responding to a second bolus of hexarelin given 120 minutes after the first (Sartorio *et al.*, 1995; Micic *et al.*, 1996).

The findings in this set of studies indicate that hexarelin will cause GH release within a period of 60 minutes. The source of this GH is presumably the readily releasable pool, since there are limited published data that suggest that hexarelin is capable of inducing GH synthesis (Locatelli *et al.*, 1994; Soto *et al.*, 1995). In addition, these data demonstrated that GHRPs were capable of stimulating GH mRNA transcription only in situations where the GHRH-dependent GH gene expression was defective. The latter was not the case in the subjects studied and reported in this thesis. Despite hexarelin's ability to stimulate GH release but not its synthesis, the magnitude of the GH secretion rates following the 60 minute hexarelin bolus was greater than that obtained following GHRH-(1-29)-NH_2.

Effect of dosing interval on the growth hormone response

GHRH-(1-29)-NH_2 boluses

The GH response to repeated boluses of GHRH-(1-29)-NH_2 in this set of studies was preserved. This contrasts with the attenuated response to the second bolus of GHRH observed by others (Massara *et al.*, 1986; Ghigo *et al.*, 1989; Arosio *et al.*, 1990; Arvat *et al.*, 1992). Possible reasons for this include different dosing regimens (dosage used and time interval between successive boluses) and/or different types of GHRH used in the various studies. The most likely reason for the discrepancy is, however, the different methods of data analysis (deconvoluted *vs* actual measured serum concentrations). Suri *et al* (1991) demonstrated that the GH secretory status at the point of administration of the second stimulus had the greatest influence on the magnitude of the response. As secretion resulting from the first bolus was still ongoing at the time the second bolus was administered this observation needs to be accounted for in subsequent analyses. Deconvoluting the data allows for this factor whereas the application of conventional statistics to compare the measured serum concentrations does not.

hexarelin and hexarelin plus GHRH-(1-29)-NH_2 boluses

The time interval between the first and the second bolus of hexarelin, alone or in combination with GHRH-(1-29)-NH_2, influenced the magnitude of the GH response. A 120 minute interval resulted in a significantly lower secretion rate. This took place even though the response to the first bolus was not significantly different from that to the 60 minute bolus, which points to a mechanism other than pool depletion. Sartorio *et al* (1995) and Micic *et al* (1996) also reported a lower GH response to the second hexarelin bolus. However, only one time interval (120 minute) was tested in their experiments. Possible mechanisms for the difference in response between the 60 minute and 120 minute interval for the hexarelin boluses, and the absence of such a phenomenon for the GHRH-(1-29)-NH_2 boluses, include: i) persistent action of first dose of hexarelin at 60 minutes but not at 120 minutes, thus augmenting the action of the second bolus given 60 minutes later, ii) time-dependent negative

feedback by increased GH and/or locally produced IGF-1 levels, iii) different cellular actions of the two secretagogues rendering the somatotroph partially refractory to the action of hexarelin during such a time interval and iv) increased somatostatin tone. These possibilities will now be discussed in more detail.

i) No data are available on the half-life of hexarelin in humans. However, since hexarelin is a small peptide, its half-life is likely to be of the order of minutes, and would be unlikely to have persistent action at 60 minutes. In addition, by using *corrected* (for explanation see Chapter 2, Studies set 1, *statistics*) peak GH secretion rates, I was able to account for this potential confounding factor. Bowers *et al* (1992 & 1993a) estimated the half-life of other GHRPs (GHRP-6 and GHRP-1) to be 15 - 20 minutes. Assuming a similar half-life for hexarelin, only 0.0625 μg/kg out of the original 1.0 μg/kg dose would be expected to be present in the circulation 60 minutes after its administration. The dose-response curves reported in this thesis suggest that such a dose (0.0625 μg/kg) would be ineffective, lending further support to above argument.

ii) The data from the GH feedback studies (set 2) in this thesis showed that hexarelin and GHRH-(1-29)-NH_2 are both subject to feedback inhibition by exogenous (and, presumably, endogenous) GH given 90 minutes after an intravenous bolus of the secretagogue without a concomitant rise in IGF-1 levels (see Chapter 3, Section 2). The generation of very high serum GH concentrations in the present set of studies (mean peak serum GH concentration following first hexarelin bolus = 85.5 mU/L; following first hexarelin plus GHRH-(1-29)-NH_2 bolus = 194.2 mU/L) and their feedback effects may account for the discrepancy in results between hexarelin and GHRH-(1-29)-NH_2. The latter produced significantly lower serum GH concentrations (mean peak serum GH concentration following first GHRH-(1-29)-NH_2 bolus = 39.9 mU/L) and demonstrated no attenuation in the GH response to repeated boluses of GHRH-(1-29)-NH_2, irrespective of the dosing interval. However, this seems a less likely explanation since GH release induced by both hexarelin and GHRH-(1-29)-NH_2 were attenuated by GH peaks of even lower magnitude (see Chapter 3, Section 2).

An alternative explanation may relate to the ability of GHRH to increase GH synthesis as well as secretion (Barinaga *et al.*, 1985; Billestrup *et al.*, 1987), and hence maintain similar magnitudes of GH secretion following the second bolus 60 or 120 minutes after the first.

iii) There is evidence that GHRH and hexarelin have different actions at the cellular level (Goth *et al.*, 1992; Akman *et al.*, 1993). GHRH-stimulated GH secretion is dependent on extracellular Ca^{2+} and may result from a cAMP-mediated influx of Ca^{2+} through voltage-dependent Ca^{2+} channels (Herrington and Hille, 1994). In contrast, GHRPs do not stimulate cAMP production, but elevate intracellular Ca^{2+} by different mechanisms (Akman *et al.*, 1993; Herrington and Hille, 1994; Bresson-Bepoldin *et al.*, 1995). The distinct modes of action of the two secretagogues are temporally dissimilar and may explain the differences observed with the dosing intervals used in this set of studies.

iv) Since SS is thought to be the physiological regulator of the GH trough periods (Tannenbaum *et al.*, 1990), it would seem plausible to attribute the attenuated GH response to the second dose of hexarelin at t = 120 minutes to increased endogenous SS release. However, the latter is unlikely to have played a major role since the response to GHRH was preserved.

Loss of synergism of hexarelin plus GHRH-(1-29)-NH_2 on repeated administration

Understanding the difference in the GH response following repeated administration of hexarelin or GHRH-(1-29)-NH_2 is of particular importance because of the observation of loss of synergism of hexarelin plus GHRH-(1-29)-NH_2 when administered on two successive occasions irrespective of the dosing interval. The effect of hexarelin plus GHRH-(1-29)-NH_2 at 60 and 120 minutes can be explained by the arithmetic sum of the two. Loss of synergism would imply either depletion or feedback inhibition of the hypothetical hypothalamic U factor, or alternatively, failure of hexarelin to cause further endogenous GHRH release. The massive GH release following the combined administration of hexarelin plus GHRH-(1-29)-NH_2 may

have partially depleted the pituitary reserves, thus limiting the amount available for further release in response to a second bolus of the two secretagogues.

Significance of 60 or 120 minute dosing intervals

From a practical point of view 60 or 120 minute dosing intervals are too short. These intervals were chosen in this study as a means of investigating the potential for the pituitary gland to respond to successive doses of hexarelin, with or without GHRH. Therapeutic programmes are likely to be based on longer dosing intervals. Further studies will need to be conducted to investigate the GH response to repeated administration of hexarelin by other routes and at longer dosing intervals, since such data are not currently available.

Summary

This set of studies shows that hexarelin is a potent GH secretagogue active after two successive doses. The GH response to hexarelin is influenced by its dosing interval. Hexarelin acts synergistically with GHRH-(1-29)-NH_2, but loses this property when administered repeatedly. These findings may have implications for the development of therapeutic regimens which are likely to entail the administration of multiple daily doses of a GHRP, alone or in combination with GHRH, in order to promote a physiological pattern of GH secretion. The low intra- and inter-individual variability in the GH response to hexarelin is advantageous if it were to be used for diagnostic purposes.

Section 2. The Effect of Growth Hormone Feedback on Hexarelin-Induced Growth Hormone Release

This set of studies, in agreement with the results of the previous set, also confirmed hexarelin as a potent intravenous GH secretagogue more effective on a weight for weight basis than GHRH-(1-29)-NH_2. It showed that the GH-releasing activity of hexarelin is subject to partial inhibition by GH itself. It also provided preliminary evidence that hexarelin is a non-specific GH secretagogue.

Growth hormone feedback

The effect of recombinant human growth hormone

The intravenous administration of 100 mU r-hGH resulted in a significantly attenuated peak serum GH response to stimulation with hexarelin or GHRH-(1-29)-NH_2. Peak serum GH concentrations following the administration of 100 mU r-hGH intravenously were of the same magnitude as the maximum physiological serum GH concentrations observed during daytime in healthy adult subjects (Brain *et al.*, 1988; Saini *et al.*, 1991). It would seem reasonable therefore to extrapolate these data and expect physiological levels of endogenous GH to exert a similar negative feedback effect.

The study design specifically addressed the effect of a GH pulse *per se*, produced in this instance by administering r-hGH, on the GH-releasing activity of hexarelin or GHRH. An alternative approach would have been the production of a GH pulse by administering a bolus of GHRH. Although the latter approach might have produced a more physiological pattern of GH response, the poor reproducibility of the GH response to GHRH would have confounded the data analysis. In addition, it might have altered hypothalamo-pituitary regulation and the state of GH releasable pools. By using a fixed dose of r-hGH, I was able to produce serum GH levels which were of similar magnitude in all the studies (10.5 ± 0.5

mU/L, 9.5 ± 1.6 mU/L and 12.0 ± 1.9 mU/L).

The bolus of secretagogue was given 90 minutes after the administration of r-hGH in order to ensure that the latter had completely cleared the circulation before endogenous GH release was stimulated. A different time interval between the two boluses might have resulted in more, or less, attenuation in the GH response to each secretagogue. In a study that was published after the execution of this set of studies, Cappa *et al* (1995) showed that exogenous GH administration did not inhibit the GH response to hexarelin. However, Cappa *et al* (1995) administered hexarelin three hours, as opposed 90 minutes, after the intravenous bolus of r-hGH. In addition, they used different doses of r-hGH (2 iU) and hexarelin (2.0 μg/kg) to those used in my studies.

Mechanism of growth hormone feedback inhibition

Different mechanisms suggested for the negative feedback effect of GH on its own secretion include stimulation of SS synthesis and release, inhibition of GHRH synthesis and release, and stimulation of local IGF-1 production from the somatotroph itself (Casanueva, 1992).

The actions of GH on the hypothalamus may occur directly through a short feedback loop, or through a long feedback loop by increasing circulating IGF-1 levels from the liver. In this set of studies, there was no rise in IGF-1 levels following the administration of r-hGH, presumably because the time interval between the two boluses was too short (Copeland *et al.*, 1980). A different study design using longer time intervals between the boluses is needed to address this issue.

GH is known to induce SS release, a mechanism that has been implicated in its negative autofeedback effect (Berelowitz *et al.*, 1981; Chihara *et al.*, 1981; Torsello *et al.*, 1988). The effect of r-hGH on stimulated GH release observed in this set of studies was probably mediated by a direct action of r-hGH on the hypothalamus to stimulate SS and inhibit GHRH production (Devesa *et al.*, 1989; Spoudeas *et al.*, 1992).

As discussed in Chapter 1 (Section 3), SS and hexarelin seem to modulate each other's actions. The differential inhibition of r-hGH on hexarelin and GHRH observed in this set of studies may be partly due to functional SS antagonism by hexarelin. The inhibition of endogenous GHRH production by r-hGH may have played a part in modifying the action of hexarelin, since they act synergistically. Which of the two mechanisms (increased SS or reduced GHRH secretion) predominates is difficult to ascertain. A reduction in serum TSH levels following the administration of r-hGH would have supported the former (Spoudeas *et al.*, 1992) but we were unable to document a significant change (mean reduction post saline = 0.2 mU/L, post r-hGH = 0.3 mU/L: p = NS).

Despite the negative feedback effect of r-hGH, hexarelin was still capable of producing supraphysiological levels of GH and remained more effective a GH secretagogue than GHRH. This suggests that hexarelin is partially refractory to the events induced by the administration of r-hGH and that its mechanism of action involves pathways additional to those affected by exogenous GH.

The effect of hexarelin on serum prolactin, cortisol and Thyroid Stimulating Hormone concentrations

The intravenous administration of 1.0 μg/kg dose of hexarelin resulted in a significant elevation in serum PRL and cortisol concentrations, indicating that the action of hexarelin was not specific for GH release. No effect on TSH concentration was observed. Detailed studies were conducted subsequently to elucidate these phenomena further. Discussion of the data relating to the PRL- and cortisol-releasing activity of hexarelin is found elsewhere in this thesis (see Discussion of the dose-response studies).

Summary

This set of studies shows that hexarelin is a potent GH secretagogue subject to partial feedback inhibition by r-hGH. This raises issues about its role (or the role of its endogenous ligand) in the physiological control of GH secretion. The studies also show that hexarelin is

a non-specific GH secretagogue. These findings may have implications for the potential use of hexarelin as a therapeutic agent.

Section 3. The Effects of Somatostatin Tone and Somatostatin Withdrawal on the Growth Hormone Response to Hexarelin, Growth Hormone Releasing Hormone and Hexarelin plus Growth Hormone Releasing Hormone

This set of studies showed that SS(1-14) attenuates the GH response to hexarelin, GHRH-(1-29)-NH_2 or both in man. Although only two doses of SS(1-14) were used, the degree of attenuation appeared to be dose related. The GH response to hexarelin was not abolished, even in the presence of a high dose (50 $\mu g/m^2/hr$) of SS(1-14), in keeping with observations by others (Arvat *et al.*, 1995). Under the same conditions of SS(1-14) inhibition, hexarelin was able to produce a greater GH response compared with GHRH-(1-29)-NH_2. Hexarelin plus GHRH-(1-29)-NH_2 had potent GH-releasing activity even in the presence of a high SS(1-14) dose of 50 $\mu g/m^2/hr$. Withdrawal of SS(1-14), on the other hand, resulted in a slight increase in the GH response to hexarelin but not that to GHRH-(1-29)-NH_2, alone or in combination with hexarelin.

Studies of somatostatin dose

Mechanism of interaction of hexarelin with somatostatin

The mechanism by which GHRPs interact with endogenous SS is unclear. There is evidence to suggest that they act as mutual functional antagonists at the hypothalamic level, since the central administration of a long acting SS analogue was able to inhibit the central action of hexarelin (Fairhall *et al.*, 1995) and hexarelin administration does not alter SS levels in the hypophysial portal blood (Guillaume *et al.*, 1994). Recent work by Dickson *et al* (1997) showed that SS was able to attenuate the GHRP-induced Fos protein expression in the

arcuate nucleus in male rats, supporting the hypothesis of functional antagonism at the hypothalamic level.

There is also evidence that hexarelin and SS act as functional antagonists at the pituitary, probably through their differential effects on somatotroph cell membrane polarisation (Pong *et al.*, 1991; Koch *et al.*, 1988). SS causes membrane hyperpolarisation (Chen and Clarke, 1992), which results from activation of an inwardly rectifying K^+ conductance (Sims *et al.*, 1991). Hyperpolarisation of the somatotroph membrane and inhibition of adenyl cyclase by SS (Koch *et al.*, 1988; Frohman *et al.*, 1992) lower intracellular Ca^{2+} and inhibit GH secretion. In contrast, as discussed in Chapter 1 (Section 3), GHRPs cause membrane depolarisation, elevation of intracellular Ca^{2+} and, subsequently, GH release.

The data from this set of studies confirm that SS and hexarelin do indeed interact to alter their respective inhibitory and stimulatory effects on GH secretion. The physiological implication of the *in vivo* interaction of SS and GHRP is that the reduction in SS tone needed to initiate a burst of GH secretion may be mediated by antagonism of its action by GHRP (or its natural ligand).

Growth hormone response to hexarelin plus GHRH-(1-29)-NH_2 in the presence of somatostatin

The greater GH response observed following the combined administration of hexarelin plus GHRH-(1-29)-NH_2 compared with the sum of the GH responses following their isolated administration under conditions of both low and high SS(1-14) tone and the magnitude of that response suggest that the combination of these two secretagogues may be a useful tool for investigating pituitary GH reserves in conditions of high SS tone, such as obesity. Indeed, GHRP plus GHRH have been found to produce massive GH release in obese subjects (Cordido *et al.*, 1993). In addition, this approach may be utilised therapeutically for reversing the GH "deficiency" states prevailing in these conditions.

Studies of somatostatin withdrawal

Effect of a single cycle of somatostatin withdrawal

The effect of SS(1-14) withdrawal compared with saline withdrawal on the GH response to either or both secretagogues was slight and insignificant, except for the response to hexarelin where the difference in response reached statistical significance. Similar findings have been reported for the GH response to GHRH-(1-29)-NH_2 administration at the time of either saline or SS(1-14) withdrawal, using doses similar to those used in my studies (Hindmarsh *et al.*, 1991). In the studies by Hindmarsh *et al* (1991), no difference in GH response was observed following one cycle of SS(1-14) withdrawal/secretagogue administration. Ho *et al* (1993) and Jaffe *et al* (1996) also found that a single cycle of SS withdrawal was ineffective at generating a GH pulse in man. However, when such cycles were repeated once or twice over, the GH responses were significantly greater than those observed following the respective repeated cycle of saline withdrawal/secretagogue administration (Hindmarsh *et al.*, 1991). The improvement seen in the GH response in the second and third cycle was thought to be due to bursts of GHRH allowing GH synthesis to take place which was then followed by the release of the synthesized and stored GH when SS(1-14) was withdrawn. Similar findings were reported by Achermann *et al* (1996), who, in addition, found that combining continuous GHRH infusion and intermittent SS(1-14) produced repeatable and significantly greater GH responses than those following SS(1-14) withdrawal alone. My studies involved only one cycle of SS(1-14) withdrawal/secretagogue administration and showed that hexarelin alone or in combination with GHRH-(1-29)-NH_2 behaved in a similar manner as GHRH-(1-29)-NH_2 alone in terms of interaction with SS(1-14) following a single cycle of infusion withdrawal.

Somatostatin withdrawal/hexarelin administration

The slight increment in GH concentration following hexarelin administration/SS20 withdrawal may reflect a small amount of endogenous GHRH release taking place following SS switch off and subsequently synergising with hexarelin. That no such effect was seen with GHRH-(1-29)-NH_2 administration/SS20 withdrawal reflects the maximal nature of the stimulus used

(Spoudeas *et al.*, 1994).

Pretreatment with either secretagogue during somatostatin infusion

A simple attempt to pretreat with either GHRH-(1-29)-NH_2 or hexarelin during a SS(1-14) infusion did not influence GH release following SS(1-14) withdrawal. Together with the findings discussed above, this supports the concept that one cycle of SS(1-14) is insufficient to allow synthesis and storage of sufficient GH to be released after SS(1-14) withdrawal. Repeated cycles would need to be performed to address this issue.

Summary

This set of studies shows that SS and hexarelin counteract their respective inhibitory and stimulatory action on GH secretion. SS(1-14) did not completely abolish the GH response to hexarelin, particularly when the latter was co-administered with GHRH-(1-29)-NH_2. The combined administration of GHRP and GHRH may have important diagnostic and therapeutic applications in conditions of high SS tone and suggest that combined therapy may be more effective in producing GH release in conditions where SS tone is unknown.

Section 4. Dose-Response Studies of the Growth Hormone, Prolactin and Cortisol Response to Hexarelin

This set of studies showed that intravenous hexarelin was capable of inducing GH release in a dose-dependent manner in healthy adult males and that hexarelin was a non-specific GH secretagogue, inducing a concomitant rise in serum PRL and cortisol levels. The rise in serum PRL was dose-related whilst that of cortisol occurred beyond a threshold dose of hexarelin. The magnitude of the GH- and cortisol-releasing activity of hexarelin had a subject effect (i.e. a high responder to a particular dose was a high responder to other doses) whilst the PRL response did not. Dose for dose, the effect of hexarelin was greater on the lactotroph compared with the somatotroph, a hitherto unreported observation which is discussed later in this Section. Intravenous hexarelin had no effect on blood sugar, serum insulin and serum TSH concentrations. The combined intravenous administration of GHRH-(1-29)-NH_2 with low dose hexarelin (0.125 μg/kg) restored its massive GH-releasing ability and resulted in only a modest elevation of PRL levels with no effect on cortisol secretion.

Growth hormone dose-response curve

The cubic model of the peak GH response *vs* hexarelin dose and the linear model using the more conventional $\log_{10}$ hexarelin dose showed that hexarelin was capable of releasing GH in a dose-dependent manner.

Maximal hexarelin dose

The GH dose-response curve (cubic model) showed a plateau at a hexarelin dose of 1.0 μg/kg, suggesting that such a dose was near-maximal. A dose-response study by Imbimbo *et al* (1994), where hexarelin doses of up to 2.0 μg/kg were administered to normal adults, showed that increasing the dose of hexarelin from 1.0 to 2.0 μg/kg resulted in only a further 6% increase in serum GH concentration, suggesting that the maximal GH response to

hexarelin occurred between 1.0 and 2.0 μg/kg. Establishing the maximal and near-maximal doses of hexarelin is important because it may have a role as a diagnostic test (Laron *et al.*, 1994), and the level is useful for comparison with other tests of pituitary GH reserve, such as the GHRH test, where the use of maximal to supramaximal doses is standard practice for testing the readily available pool of GH (Spoudeas *et al.*, 1994).

High dose *vs* low dose hexarelin

Most studies of hexarelin have been conducted using high doses (1.0 μg/kg) which are known to induce massive GH release. Such GH levels are not needed for normal linear growth. Doses of hexarelin which are capable of generating lower but adequate levels of GH in the blood are more likely to be used in therapy, since they would mimic the physiological levels of serum GH essential for normal growth. The GH dose-response curve shows that doses of hexarelin as low as 0.2 μg/kg are capable of generating serum GH concentrations equivalent to the maximum seen in normal adults during day time (Brain *et al.*, 1988; Saini *et al.*, 1991), and would be expected to generate at least similar levels in children.

Subject effect on the growth hormone response to hexarelin

The data showed that there was a significant subject effect on the magnitude of the peak GH response to various doses of hexarelin, namely that subjects who had a large GH response to a particular hexarelin dose were likely to be high responders to other doses, and, similarly, subjects who had a low GH response to a particular hexarelin dose were likely to be low responders to other doses. This finding may have implications for the potential use of hexarelin as a diagnostic agent. As well as establishing that an individual is capable of responding to a particular dose of hexarelin, the magnitude of the response would be an important observation. It would help predict the nature of the response (high or low) to different doses, and may be an important consideration when selecting subjects for treatment with hexarelin. The magnitude of the response may also help determine the dose of hexarelin to be used for a particular individual.

Prolactin and cortisol dose-response curves

The data showed that hexarelin was capable of inducing PRL release in a clear dose-dependent manner, in a similar manner to its GH-releasing effect. The effect on cortisol release was more obvious beyond a threshold dose of hexarelin (0.5 μg/kg), as shown (Figure 32) by the step increase in the maximum change in serum cortisol concentration.

Non-specificity of hexarelin action

The concomitant rise in serum PRL and cortisol seen with intravenous hexarelin indicates, contrary to earlier beliefs (Bowers *et al.*, 1984; Ilson *et al.*, 1989), that GHRPs are not as specific GH secretagogues as was first thought. Other workers have documented this non-specificity of hexarelin and GHRP-6 and found that the cortisol release was accompanied by a rise in ACTH (Frieboes *et al.*, 1995; Korbonitz *et al.*, 1995). These data suggest that hexarelin acts at receptor sites not purely confined to the somatotroph. The PRL-releasing effect of hexarelin indicates that GHRPs stimulate the lactotroph, possibly by a direct action. Action at a supra-pituitary level by altering the dopaminergic control of the anterior pituitary is also possible. The absence of hexarelin effect on serum TSH levels, observed in this set of studies and reported by others (Korbonits *et al.*, 1995), argues against the effect on PRL being TRH mediated.

The cortisol- and concomitant ACTH-releasing effect of hexarelin (Frieboes *et al.*, 1995; Korbonitz *et al.*, 1995) may reflect stimulation of ACTH release directly by action at the pituitary or indirectly by stimulating the release of corticotrophin releasing factor. GHRPs may, in addition, stimulate cortisol release by a direct action on adrenocortical cells, though evidence for this is currently lacking.

Significance of the prolactin- and cortisol-releasing effect of hexarelin

Although the maximum levels to which serum PRL and cortisol concentrations rose remained within the normal range and the values returned to baseline levels by 120 minutes, these

findings may have far reaching implications in terms of the potential use of GHRPs in the therapeutic setting. The prolonged and repeated use of GHRPs may result in hypercortisolaemic and/or hyperprolactinaemic states if such effects on PRL and cortisol release were sustained.

In an attempt to begin to address the issue of the effect of chronic hexarelin administration on serum PRL and cortisol, I measured the serum concentrations of these two hormones in the samples collected from the first set of studies (The effect of single and repeated doses of hexarelin, GHRH and hexarelin plus GHRH on GH secretion). The idea behind this exercise was to examine the effect of repeated doses (two, in this case) of hexarelin on serum PRL and cortisol concentrations. The data were analysed on the basis of the maximum change in serum concentration compared to baseline values at the time of hexarelin administration ($t \approx 0$ minutes or $t = 120$ minutes). Only the effects of the second bolus given 120 minutes (and not 60 minutes) after the first were studied, since the serum PRL and cortisol concentrations had returned to baseline by this time. The intravenous administration of a second bolus of hexarelin 120 minutes after the first resulted in significant elevation of the serum PRL and cortisol concentrations compared to saline ($p < 0.05$); the maximum change in serum concentration for each hormone was similar in magnitude after each of the hexarelin boluses, as shown in the Table 22. These findings confirm the need to investigate whether the PRL- and cortisol-releasing activity of hexarelin and other GHRPs is sustained during chronic use.

Table 22. Maximum change in serum PRL and cortisol concentrations following the intravenous administration of two boluses of hexarelin or saline 120 minutes apart.

nature of i.v. bolus	maximum change in serum PRL (mean ± SEM)	maximum change in serum cortisol (mean ± SEM)
1st hexarelin at t = 0 min	176.4 ± 22.8 mU/L*	60.7 ± 25.8 nmol/L**
2nd hexarelin at t = 120 min	112.8 ± 26.9 mU/L*	127.2 ± 19.0 nmol/L**
1st saline at t = 0 min	-24.2 ± 12.7 mU/L	-55.0 ± 40.0 nmol/L
2nd saline t = 120 min	17.3 ± 18.3 mU/L	-49.5 ± 29.8 nmol/L

* $p > 0.05$

** $p > 0.05$

The dose-response study has only addressed the PRL- and cortisol-releasing activity of hexarelin in the acute setting so the effects of chronic administration of hexarelin on serum PRL and cortisol remain unknown. It is prudent to mention that, thus far, there have been no reports of an orally administered GHRP resulting in significant PRL or cortisol release (Bowers *et al.*, 1992; Bowers, 1994). The reasons for this are unclear but may be related to the low bioavailability of GHRPs following oral ingestion (Bowers, personal communication).

The relative activity of hexarelin on the somatotroph and lactotroph

The maximum change in serum GH and PRL from baseline values, expressed in molar terms and plotted against $\log_{10}$ transformed hexarelin dose showed that the linear relationship for PRL was steeper than that for GH. This indicates that, at any given dose, hexarelin was capable of releasing more PRL than GH. However, the linear relationships of $\log_{10}$ hexarelin dose *vs* maximum change in PRL and GH were not parallel. This suggests that the kinetics of the hexarelin-induced PRL and GH release are different. Limited conclusions can be drawn from this observation, since other confounding factors were not studied. For example, changing the somatostatin and/or dopaminergic tone might have altered the relative activity of hexarelin on the lactotroph and somatotroph. Whilst, for research purposes, the somatostatin tone can be varied (increased or abolished) with relative ease in humans, it is much more difficult to abolish the dopaminergic system in the central nervous system. Animal studies may be the way forward for studying this phenomenon.

Low dose hexarelin, with or without growth hormone releasing hormone

The finding that the hexarelin-induced rise in serum PRL and cortisol is dose-dependent, coupled with its dose-dependent GH release, has enabled me to identify a dose of hexarelin (0.2 μg/kg) which results in adequate GH release with minimal effects on serum cortisol and PRL.

The synergistic GH-releasing effect of GHRH plus GHRP, using near-maximal doses, is well documented and has been discussed already in this thesis. The use of low dose GHRP with

maximal dose of GHRH is also known to be synergistic (Bowers *et al.*, 1992). This study shows that the combination of GHRH-(1-29)-NH_2 (1.0 μg/kg) and low dose hexarelin (0.125 μg/kg) induces massive GH release with no effect on serum cortisol and only a modest elevation of serum prolactin levels. Furthermore, using a combination of GHRH and low dose hexarelin not only restores but exceeds the massive GH release observed with near-maximal doses of hexarelin alone. This opens up the possibility of using even smaller doses of GHRP in combination with GHRH.

The synergistic effect of GHRH plus low dose hexarelin on GH but not PRL and cortisol release suggests that the mechanism by which synergism occurs is independent of the pathways which control PRL and cortisol release. Since hexarelin alone stimulates pathways which induce GH, PRL and cortisol release, and GHRH alone stimulates pathways which induce GH and PRL release (see Chapter 3, Section 2), it would seem likely that a third pathway operates to bring about specific GH synergism. The postulated U factor discussed in Chapter 1 (Section 3) and this Chapter (Section 1) could account for this phenomenon, on the basis that U factor has specific action on the somatotroph without affecting lactotrophs and corticotrophs. The hypophysiotropic factor Pituitary Adenylate Cyclase-Activating Polypeptide (PACAP) is a possible candidate for this U factor on account of its ability to release GH, but not PRL, in man (Rawlings and Hezareh, 1996). However, there is evidence that PACAP can also influence ACTH release *in vivo* (in rats) (Leonhardt *et al.*, 1992). Further studies are needed before a definitive statement can be made about the role, if any, of PACAP in the synergistic GH-releasing activity of GHRP plus GHRH.

The combined use of GHRP and GHRH in the therapeutic setting has many advantages. The data reported in this thesis show that, given together, they induce massive GH release, which persists on successive administration (despite loss of synergism) and also in the presence of high somatostatin tone. By using low doses of hexarelin, the undesirable PRL- and cortisol-releasing effects are minimised. Since GHRPs act mainly to promote GH secretion as opposed to synthesis, while GHRH enhances both GH synthesis and release, it can be postulated that combined therapy would avoid the potential problem of depletion of pituitary GH reserves.

Although the combined use of GHRH and GHRP is an attractive therapeutic option, the route and frequency of administration of the two secretagogues need to be established. Since GHRPs are active following oral ingestion, this would seem the obvious route for their administration. GHRH, on the other hand, would need to be given parenterally, possibly as a depot preparation once monthly. The dosing regimen for GHRP would be twice or three times daily. Clearly, further studies need to be conducted to validate this therapeutic regimen.

Summary

This study provides important dose-response data for hexarelin's GH-, prolactin- and cortisol-releasing properties. It provides further confirmation of the synergistic action of low dose GHRP plus GHRH and the potential advantage of applying this approach in future therapeutic trials.

CONCLUSION

The physiological studies in this thesis have provided data which can be applied to understand the potential role of GHRPs in the control of GH secretion. The data can also be utilised in the clinical applications of GHRPs.

GHRPs are small synthetic peptides capable of inducing GH release in a magnitude greater than that reported for any other GH secretagogue. The studies in this thesis examined the properties of a novel GHRP, hexarelin, which differs from the "native" GHRP-6 in that the second tryptophan derivative has been methylated. This confers added physical stability to the peptide and seems beneficial for its GH releasing activity. Preliminary studies showed that it was indeed capable of inducing substantial GH release in man.

Intravenous hexarelin is a potent GH secretagogue and is well tolerated in man. Its GH-releasing action is repeatable and reproducible. Hexarelin acts synergistically with GHRH to produce massive GH release, by an unclear mechanism which may involve an, as yet, unidentified factor or pathway. The kinetics of the GH response to intravenous hexarelin, GHRH or both together are similar. Successive administration of hexarelin, alone or in combination with GHRH, results in further significant GH release. However, the magnitude of the GH response to repeated GHRP administration seems to be time interval dependent. This phenomenon is likely to be multifactorial involving negative feedback by the released GH itself, either directly and/or by causing an increase in somatostatin tone and local IGF-1 production. The synergistic GH-releasing phenomenon of hexarelin plus GHRH is lost on repeated administration. The mechanism for this phenomenon is obscure. It may involve GH depletion, or depletion of the hypothetical factor which brings about synergism. Despite the loss of synergism, the magnitude of the GH response following repeated administration of hexarelin plus GHRP remains substantial.

The GH response to hexarelin is subject to feedback inhibition by exogenous GH. Thus, it is likely that the putative endogenous GHRP ligand would be subject to feedback inhibition

by the endogenous GH released as a result of its action on the somatotroph. The feedback inhibition does not seem to be mediated by peripherally derived IGF-1, though locally produced IGF-1 may play a part in this phenomenon. The inhibition may well be related to increased somatostatin release, which attenuates the GH response to hexarelin but does not completely abolish it. The latter finding is in agreement with the hypothesis that GHRPs and somatostatin are functional antagonists. The massive GH release induced by hexarelin and GHRH is also attenuated by increasing somatostatin dose, though the combination of the two secretagogues remains the most potent inducer of GH release known in situations of high somatostatin tone.

Although somatostatin withdrawal does not alter the kinetics of the GH response to hexarelin, alone or in combination with GHRH, it does augment the magnitude of the GH response to hexarelin alone. This probably reflects GHRP-induced endogenous GHRH release, which has remained quiescent during the state of high somatostatin tone. Interestingly, simple pretreatment with hexarelin *during* a somatostatin infusion failed to influence GH release when somatostatin was withdrawn. These findings, together with those reported by others, indicate the need for repeated cycles of infusion/withdrawal to investigate fully this aspect of GHRP physiology.

The actions of hexarelin and other GHRPs are not specific. In addition to stimulating the somatotroph to release GH, they also stimulate the lactotroph and the corticotroph, resulting in prolactin and cortisol release. The GH, prolactin and cortisol responses to hexarelin are dose-related. At higher doses of hexarelin (1.0 μg/kg) all three hormones are released in a potent manner. At lower doses (0.125 μg/kg), the concomitant and undesirable prolactin and cortisol release is minimal. Moreover, the combination of low dose hexarelin (0.125 μg/kg) plus GHRH (1.0 μg/kg) is synergistic for GH release, but not for prolactin and cortisol. Thus, the latter approach may be utilised therapeutically to maximise GH replacement, avoiding the undesirable releasing effects of GHRPs on other pituitary hormones. The finding that some subjects show a persistently larger GH response to increasing doses of hexarelin may be useful in selecting individuals likely to benefit from this form of treatment. Finally, the dose-response studies showed that hexarelin has no effect on serum insulin, thyroid stimulating hormone or

blood glucose.

So how do the above findings add to our current state of understanding of growth hormone regulation? The findings strongly suggest that GHRPs have an important role in growth hormone physiology. Clearly, GHRPs are potent inducers of GH secretion. If an endogenous GHRP-like ligand were to exist, the physiology of GH regulation would have to be redefined. The greater potency of GHRPs compared to GHRH could imply that they, and not GHRH, are the prime endogenous stimulators of GH secretion. The ability of the somatotroph to respond to repeated stimulation by GHRP lends further support to the notion that GHRPs play a major role in GH secretion. A negative feedback mechanism exists which keeps "in check" the amount of GH released in response to GHRP. This involves GH itself, acting directly or indirectly via SS. The variability of endogenous SS tone would also control the amount of GH released in response to GHRP. This is supported by the findings that SS attenuates the GH response to GHRP, and that the magnitude of the GH response to repeated GHRP administration is time interval dependent. Conversely, the magnitude of the GH response to GHRP could be increased by SS withdrawal, possibly through GHRH release synergising with GHRP. The precise physiological relevance of the synergistic action of GHRP plus GHRH is unclear, though it may play a role in determining the amplitude of the GH pulse at times of high SS tone. The interaction of GHRP, GHRH and SS is complex and may well involve yet another pathway or factor. The effects of GHRP on non-somatotroph cells (corticotrophs and lactotrophs) suggest that GHRPs act through multiple pathways.

The studies reported in this thesis provide new data on the properties of GHRPs, their mode of action and their interaction with the main regulators of GH secretions. These data supplement the already reported data on GHRPs in a way to improve our understanding of the potential physiological role of GHRPs in the control of GH production and secretion. Since no endogenous GHRP-like ligand has been identified yet, the implications of these findings on the physiological control of GH secretion remain speculative. However, the GHRP data accumulated so far and, in particular, the recent cloning of the GHRP receptor strongly support the existence of such a ligand.

Whether or not an endogenous GHRP-like ligand exists and whether or not it has a physiological role in growth hormone regulation, the findings reported here may still have important implications for the clinical applications of GHRPs, in both the diagnostic and therapeutic setting. Clearly, the indications for using GHRPs as a diagnostic or therapeutic tool will need to defined.

APPENDICES

Appendix 1. Data for the peak serum GH concentration (mU/L) in response to a single bolus at t = 0 minutes of saline, hexarelin, GHRH-(1-29)-NH_2 or hexarelin plus GHRH-(1-29)-NH_2, using doses of 1.0 micrograms/kg of secretagogue(s). The arithmetic sum of the peak serum GH concentrations following the isolated administration of hexarelin and GHRH-(1-29)-NH_2 is also given. Study design is shown in Figure 6.

Subject	Peak serum GH concentration (mU/L) following bolus of:				
	saline (study no. 1)	hexarelin (study no. 2)	GHRH (study no. 5)	hexarelin + GHRH (study no. 8)	arithmetic sum (study no. 2 & 5)
RR	21.0	65.7	116.3	246.6	182.0
PL	0.6	50.2	13.3	67.5	63.5
RH	0.8	35.6	5.0	74.5	40.6
SD	9.8	87.6	29.8	126.1	117.4
RT	0.9	83.0	75.8	286.2	158.8
MH	1.2	65.6	133.7	217.0	199.3
	xxx	hexarelin	GHRH	hexarelin + GHRH	arithmetic sum
	xxx	(study no. 3)	(study no. 6)	(study no. 9)	(study no. 3 & 6)
RR	xxx	70.4	34.1	176.0	104.5
PL	xxx	43.0	72.1	141.6	115.1
RH	xxx	73.3	3.3	90.0	76.6
SD	xxx	158.8	24.3	198.4	183.1
RT	xxx	84.2	6.9	351.6	91.1
MH	xxx	70.7	10.5	190.8	81.2
	xxx	hexarelin	GHRH	hexarelin + GHRH	arithmetic sum
	xxx	(study no. 4)	(study no. 7)	(study no. 10)	(study no. 4 & 7)
RR	xxx	76.9	56.7	151.0	133.6
PL	xxx	52.3	22.4	117.6	74.7
RH	xxx	90.9	12.2	88.6	103.1
SD	xxx	76.5	24.3	258.2	100.8
RT	xxx	219.4	7.8	439.6	227.2
MH	xxx	135.2	70.6	272.2	205.8

Appendix 2. Peak GH secretion rates following bolus intravenous administration of saline, hexarelin, GHRH-(1-29)-NH_2, or hexarelin plus GHRH-(1-29)-NH_2 given 60 or 120 minutes apart. Study design is shown in Figure 6.

Subject	Study number	Peak GH secretion rates (mU/min) following bolus(es) given at:		
		t = 0 min	t = 60 min	t = 120 min
RR	1	32.0	0.0	1.0
PL	(saline at t = 0 min,	1.1	1.1	0.3
RH	saline at t = 60 min,	1.4	1.4	1.5
SD	saline at t = 120 min)	5.5	1.4	2.7
RT		3.1	1.3	0.9
MH		3.0	1.4	11.7
RR	2	167.6	27.7	2.4
PL	(hexarelin at t = 0 min,	142.0	5.0	2.8
RH	saline at t = 60 min,	126.2	27.3	0.0
SD	saline at t = 120 min)	242.7	127.1	12.7
RT		249.4	26.0	1.7
MH		288.1	46.1	4.0
RR	3	172.8	182.4	62.7
PL	(hexarelin at t = 0 min,	159.7	75.7	0.7
RH	hexarelin at t = 60 min,	245.0	140.8	39.7
SD	saline at t = 120 min)	432.9	453.2	73.5
RT		269.7	335.5	7.1
MH		301.9	144.9	13.1
RR	4	195.3	26.6	132.9
PL	(hexarelin at t = 0 min,	158.5	2.9	13.7
RH	saline at t = 60 min,	252.8	5.2	94.7
SD	hexarelin at t = 120 min)	213.6	98.7	160.9
RT		861.6	103.2	186.1
MH		304.1	44.6	161.1
RR	5	410.6	37.0	9.7
PL	(GHRH at t = 0 min,	37.4	2.3	1.9
RH	saline at t = 60 min,	21.6	9.1	1.4
SD	saline at t = 120 min)	120.2	32.4	3.4
RT		166.1	166.9	14.7
MH		550.5	132.3	21.3
RR	6	107.7	63.4	17.0
PL	(GHRH at t = 0 min,	197.7	220.3	38.2
RH	GHRH at t = 60 min,	14.0	11.5	3.7
SD	saline at t = 120 min)	103.9	136.8	14.5
RT		7.8	22.2	102.2
MH		46.2	46.2	10.2
RR	7	121.8	89.9	375.6
PL	(GHRH at t = 0 min,	50.3	25.1	13.9
RH	saline at t = 60 min,	53.3	1.4	4.9
SD	GHRH at t = 120 min)	89.5	34.5	89.7
RT		33.7	34.9	239.1
MH		226.4	12.9	34.4

Appendix 2 continued overleaf

Appendix 2 (cont.)

Subject	Study number	Peak GH secretion rates (mU/min) following bolus(es) given at:		
		t = 0 min	t = 60 min	t = 120 min
RR	8	743.1	196.1	14.8
PL	(hexarelin + GHRH	260.6	3.3	2.0
RH	at t = 0 min,	266.3	78.7	0.0
SD	saline at t = 60 min,	328.4	192.0	11.5
RT	saline at t = 120 min)	917.8	35.5	4.7
MH		729.6	110.2	25.5
RR	9	475.9	377.6	185.9
PL	(hexarelin + GHRH	372.6	415.8	18.0
RH	at t = 0 min,	266.8	179.9	54.2
SD	hexarelin + GHRH	714.4	494.0	61.9
RT	at t = 60 min,	1056.8	687.6	120.2
MH	saline at t = 120 min)	611.4	664.8	80.6
RR	10	389.7	265.7	164.9
PL	(hexarelin + GHRH	455.2	118.1	97.1
RH	at t = 0 min,	320.5	84.4	120.0
SD	saline at t = 60 min,	691.6	425.0	400.1
RT	hexarelin + GHRH	1501.1	642.7	431.7
MH	at t = 120 min)	1210.0	89.5	377.0

Appendix 3. Data (serum GH concentrations in mU/L) for the effect of r-hGH on the GH response to hexarelin or GHRH-(1-29)-NH_2. Study design is shown in Figure 7.

Subject	Sample time (min)	Serum GH concentration (mU/L)					
		Study 1 (sal/sal)	Study 2 (sal/hex)	Study 3 (sal/GHRH)	Study 4 (r-hGH/sal)	Study 5 (r-hGH/hex)	Study 6 (r-hGH/GHRH)
PL	-60	0.9	3.5	0.5	6.6	0.5	2.3
	-45	0.9	4.5	0.6	4.5	0.5	1.6
	-30	1.0	3.4	0.5	3.0	0.5	1.1
	-15	1.1	1.3	0.5	2.1	0.5	0.7
	0	1.3	0.9	0.5	1.3	0.5	0.7
	5	1.4	0.8	1.7	11.7	0.7	9.4
	10	1.5	0.5	0.7	5.8	3.9	3.6
	15	1.5	0.5	0.5	4.0	3.6	1.9
	30	1.5	0.5	0.9	1.9	2.4	1.1
	45	0.9	0.5	0.5	0.8	1.9	0.8
	60	1.1	0.5	0.5	0.5	2.6	0.5
	75	0.5	0.5	0.5	0.5	2.3	0.5
	90	0.6	0.5	0.5	0.5	1.9	0.5
	105	0.6	22.7	11.3	0.5	33.3	1.2
	120	0.5	27.4	7.1	0.5	43.0	1.8
	135	0.5	23.3	5.1	0.5	32.3	3.3
	150	0.5	17.3	3.8	0.5	21.4	2.5
	165	0.7	9.5	4.9	0.5	10.2	1.7
	180	0.7	5.4	9.8	0.5	6.0	1.3
SD	-60	0.5	0.5	0.8	0.5	0.5	31.6
	-45	0.6	0.5	0.8	0.5	0.5	31.2
	-30	3.4	0.5	0.5	0.5	0.5	25.2
	-15	2.9	0.5	0.5	0.5	0.5	23.4
	0	8.3	0.5	0.7	0.5	1.0	8.9
	5	6.3	0.5	0.5	17.6	7.0	18.0
	10	5.5	0.7	0.5	6.6	3.8	14.4
	15	6.4	1.0	0.5	4.5	3.8	10.9
	30	5.0	1.0	0.5	1.7	2.5	7.5
	45	2.5	1.3	0.8	0.9	6.4	4.5
	60	1.8	0.7	0.7	0.8	13.4	2.6
	75	1.1	0.6	0.6	0.5	15.3	1.6
	90	0.9	0.9	0.5	0.5	14.2	1.6
	105	1.4	44.6	51.7	0.5	64.2	4.5
	120	0.8	104.5	90.6	0.5	117.9	10.2
	135	2.0	188.2	82.2	0.5	79.5	10.4
	150	1.4	160.4	78.0	0.5	62.4	11.0
	165	0.9	93.3	71.7	0.5	42.6	10.2
	180	0.9	72.6	62.2	0.5	30.9	9.1
RT	-60	0.5	1.6	1.0	3.6	1.4	0.6
	-45	0.5	1.1	0.5	7.7	0.8	0.5
	-30	0.5	0.5	0.5	3.5	0.8	0.5
	-15	0.5	0.5	0.5	2.2	0.7	0.5
	0	0.5	0.6	0.5	1.4	0.5	0.5
	5	0.5	0.6	0.5	12.1	6.9	13.0
	10	0.5	1.2	0.5	6.3	3.6	6.6
	15	0.5	3.3	0.5	5.9	3.9	4.6
	30	0.5	11.9	0.5	3.0	11.3	2.6
	45	0.5	20.8	0.9	1.7	8.3	1.6
	60	0.5	22.7	0.5	3.7	3.2	0.7
	75	0.5	9.9	0.5	16.8	1.3	0.7
	90	0.5	5.0	0.5	19.6	0.7	0.5
	105	0.5	100.0	18.7	13.5	27.6	35.9
	120	0.5	155.0	64.8	8.5	51.1	53.0
	135	0.5	172.6	56.3	4.0	47.4	77.7
	150	1.0	88.4	32.4	3.0	30.2	68.5
	165	5.6	83.6	21.9	1.6	21.1	43.2
	180	8.4	50.3	9.8	1.2	12.5	25.2

Appendix 3 (cont.)

Subject	Sample time (min)	Serum GH concentration (mU/L) Study 1 (sal/sal)	Study 2 (sal/hex)	Study 3 (sal/GHRH)	Study 4 (r-hGH/sal)	Study 5 (r-hGH/hex)	Study 6 (r-hGH/GHRH)
PJ	-60	0.5	0.5	0.5	1.0	0.7	0.7
	-45	0.7	0.6	0.5	0.8	0.5	3.6
	-30	0.5	0.6	0.5	0.6	0.5	3.1
	-15	0.7	0.6	0.5	0.5	0.5	1.8
	0	0.5	0.6	0.5	0.5	0.8	1.0
	5	0.5	0.6	0.5	9.4	7.6	12.6
	10	0.5	0.6	0.5	4.4	4.7	9.6
	15	0.5	0.5	0.5	3.3	2.4	8.6
	30	0.7	0.5	0.5	1.8	1.2	8.3
	45	0.5	0.5	0.5	1.8	1.0	3.6
	60	0.5	0.9	3.5	2.4	2.1	1.8
	75	0.7	1.0	10.6	3.8	3.6	1.4
	90	0.7	0.8	7.7	2.6	1.9	0.7
	105	1.9	64.9	16.4	1.4	49.4	10.1
	120	2.3	106.8	9.3	1.0	92.4	11.1
	135	1.4	187.4	6.7	0.6	88.7	7.4
	150	0.9	132.9	6.2	0.5	80.8	4.5
	165	0.8	76.5	5.0	0.5	63.7	2.5
	180	0.5	51.1	3.3	0.5	38.0	1.6
JT	-60	0.5	0.5	0.6	0.5	0.5	0.5
	-45	0.5	0.5	0.5	0.7	0.5	1.3
	-30	0.5	0.5	0.5	0.5	0.5	5.3
	-15	0.5	0.5	0.5	0.5	0.5	6.4
	0	0.5	0.5	0.5	0.7	0.5	4.0
	5	0.5	0.5	0.5	11.0	17.3	14.8
	10	0.5	0.5	0.5	7.5	11.1	8.0
	15	0.5	0.5	0.5	5.7	5.7	5.7
	30	0.5	0.5	0.5	1.8	2.6	2.6
	45	0.5	0.5	0.5	1.0	1.1	1.3
	60	0.8	0.5	0.5	0.7	0.8	0.6
	75	1.4	0.5	0.5	0.6	0.5	0.5
	90	2.8	0.5	0.5	0.5	0.5	0.5
	105	1.2	15.7	27.6	0.5	26.4	30.3
	120	2.2	62.3	44.1	0.6	111.6	46.1
	135	0.6	65.8	55.5	0.7	103.8	38.0
	150	0.5	61.9	76.5	0.6	68.1	22.0
	165	0.5	57.1	70.0	1.5	45.8	15.1
	180	0.5	38.2	41.5	1.5	24.6	10.1
AJ	-60	0.5	0.5	0.5	0.5	0.5	0.5
	-45	0.5	1.0	0.5	0.5	0.5	0.5
	-30	0.5	0.9	0.5	0.5	0.5	0.5
	-15	0.5	0.5	0.5	0.5	0.5	0.5
	0	0.5	0.7	0.9	0.5	0.7	0.5
	5	0.5	0.6	3.1	8.3	10.4	9.0
	10	0.5	0.5	0.9	4.9	5.7	6.0
	15	0.5	0.6	0.9	3.1	3.4	3.4
	30	0.5	0.8	0.5	1.4	1.8	1.6
	45	0.5	0.6	0.5	0.5	1.3	0.9
	60	0.5	0.5	0.5	0.5	0.9	0.5
	75	0.5	0.6	0.5	0.5	0.5	0.5
	90	0.5	1.1	0.5	0.5	1.3	0.5
	105	0.5	71.2	3.1	0.5	64.6	4.1
	120	0.5	94.4	8.2	0.5	82.5	6.4
	135	0.5	82.3	7.8	0.5	57.3	7.9
	150	0.5	58.1	13.4	0.5	40.3	6.0
	165	0.5	24.3	13.0	0.5	25.3	3.2
	180	0.5	16.4	9.7	0.5	15.0	1.1

sal: saline, hex: hexarelin

First bolus was administered at t = 0 min and the second at t = 90 min.

Appendix 4. Serum IGF-1 concentrations before (at t = 0 minutes) and 90 minutes after the administration of saline or r-hGH.

Subject	Sample time (min)	Serum IGF-1 concentration (micrograms/L)					
		Study 1 (sal/sal)	Study 2 (sal/hex)	Study 3 (sal/GHRH)	Study 4 (r-hGH/sal)	Study 5 (r-hGH/hex)	Study 6 (r-hGH/GHRH)
PL	0	174.2	134.6	198.0	198.0	162.4	140.6
	90	126.7	154.4	202.0	174.2	162.4	180.2
SD	0	237.6	206.0	206.0	184.1	166.3	174.2
	90	206.0	206.0	186.1	180.2	176.2	198.0
RT	0	374.2	433.6	475.2	340.6	401.9	340.6
	90	364.3	352.4	445.5	336.6	378.2	382.1
PJ	0	176.2	134.6	154.4	156.4	176.2	164.3
	90	229.7	184.1	186.1	146.5	160.4	176.2
JT	0	267.3	257.4	318.8	285.1	257.4	269.3
	90	257.4	207.9	281.2	253.4	283.1	207.9
AJ	0	196.0	200.0	188.1	205.9	134.6	231.7
	90	227.7	229.7	170.3	239.6	160.4	217.8

sal: saline
hex: hexarelin

Appendix 5. Data (serum GH concentration at each sampling point) for the effect of somatostatin dose on the GH response to hexarelin, GHRH-(1-29)-NH_2 or both. Study design is shown in Figure 8.

Subject	Study	Serum GH concentration (mU/L)										
	number	t = -30	t = -15	t = 0	t = 15	t = 30	t = 45	t = 60	t = 75	t = 90	t = 105	t = 120
RR	1	18.3	27.2	24.5	21.0	16.7	8.0	4.9	2.4	1.7	1.8	0.9
MH	(saline	1.2	1.2	1.3	1.2	0.5	0.6	0.5	0.5	0.5	0.5	1.0
PL	infusion;	0.8	0.6	0.6	0.5	0.5	0.5	0.6	0.5	0.5	0.6	1.1
RH	saline bolus)	0.6	0.8	1.0	0.7	0.5	0.5	0.8	0.5	0.5	0.5	0.5
RR	2	1.1	3.3	2.3	8.6	48.7	65.7	52.7	34.5	23.2	12.4	8.5
MH	(saline	4.0	3.2	2.8	47.0	65.6	46.6	25.2	23.3	15.9	8.3	4.1
PL	infusion;	1.2	0.9	0.5	23.1	48.4	50.2	33.8	18.7	8.0	5.1	3.7
RH	hex bolus)	0.5	0.5	0.5	3.8	30.0	35.6	25.6	19.3	8.4	4.4	1.9
RR	3	8.0	13.9	15.0	116.3	108.8	87.6	51.4	36.3	19.6	14.8	8.8
MH	(saline	1.3	1.1	2.0	123.7	133.7	126.1	91.9	76.5	60.8	42.3	27.2
PL	infusion;	1.0	1.0	0.5	9.8	13.3	11.0	6.1	2.3	1.8	1.5	1.4
RH	GHRH bolus)	0.5	0.5	0.5	5.0	4.4	3.9	4.0	4.0	2.4	1.8	1.8
RR	4 (saline	0.8	1.3	1.3	97.1	246.6	171.0	192.4	106.5	106.8	80.5	52.9
MH	infusion;	1.7	1.4	1.0	163.2	198.0	217.0	166.6	110.5	69.4	52.9	28.3
PL	hex + GHRH	0.5	0.5	0.5	67.2	67.5	61.3	41.8	22.4	10.2	5.8	3.7
RH	bolus)	0.5	0.5	0.5	59.6	74.3	64.8	59.6	48.3	35.8	24.0	9.9
RR	5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5
MH	(SS20	1.0	0.7	0.6	0.8	1.4	0.6	0.7	0.7	0.7	0.7	0.8
PL	infusion;	2.9	1.3	1.2	1.3	1.0	0.7	0.9	0.1	0.7	0.8	0.5
RH	saline	0.7	0.5	0.5	0.5	0.5	0.5	0.6	0.5	0.5	0.5	0.5
KW	bolus)	1.2	0.9	1.0	1.1	0.9	1.0	0.8	0.6	0.8	0.7	0.8
AD		1.2	1.2	0.6	0.7	0.6	0.7	0.7	0.7	0.5	0.7	0.7
RR	6	1.0	1.1	0.7	15.3	50.9	54.8	39.0	27.5	16.7	10.2	5.4
MH	(SS20	3.7	2.1	1.5	1.8	8.8	18.0	13.6	10.5	4.8	2.6	1.5
PL	infusion;	0.5	0.7	0.6	4.7	5.7	4.9	2.7	1.6	1.1	0.8	1.3
RH	hex	0.5	0.5	0.5	18.6	39.7	31.2	21.4	10.0	5.9	2.4	1.6
KW	bolus	1.0	0.5	0.5	6.5	30.0	19.9	16.9	10.8	5.6	5.0	1.0
AD		0.7	0.6	0.6	14.8	24.2	10.8	6.3	2.3	1.6	1.8	0.9
RR	7	0.9	0.6	0.6	0.9	1.4	2.8	3.1	4.0	3.6	2.2	1.6
MH	(SS20	3.8	1.5	1.4	24.7	26.7	14.5	8.4	5.4	2.8	1.5	1.5
PL	infusion;	4.4	2.0	1.3	1.6	2.0	1.6	1.7	1.8	1.3	0.9	1.3
RH	GHRH	0.5	0.5	0.5	5.5	3.3	3.3	3.1	2.3	1.9	1.2	1.0
KW	bolus)	0.7	1.0	1.1	2.3	2.2	2.1	2.1	2.2	2.0	1.9	1.6
AD		0.5	0.5	0.5	13.2	26.7	24.5	11.5	6.9	5.7	2.4	1.5
RR	8	0.5	0.5	0.5	88.2	134.9	190.0	159.6	102.4	64.0	41.1	24.1
MH	(SS20	0.5	0.6	0.4	114.4	107.6	111.0	101.7	63.9	49.7	35.5	21.1
PL	infusion;	0.9	1.2	1.5	83.2	94.8	77.5	59.0	33.4	22.2	11.7	5.4
RH	hex + GHRH	0.6	0.5	0.5	109.3	100.4	108.1	76.5	49.4	23.6	15.9	8.4
KW	bolus)	0.5	0.5	0.5	29.6	20.1	17.0	15.1	9.9	5.8	2.7	2.1
AD		0.5	0.6	0.5	53.2	39.1	35.4	36.3	27.5	17.6	11.6	8.2
RR	9	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5
MH	(SS50	5.7	1.8	1.2	0.8	0.7	0.7	0.5	0.5	0.5	3.0	0.5
PL	infusion;	0.5	0.7	0.5	0.6	0.6	0.6	0.7	0.9	0.5	0.5	0.7
RH	saline	1.0	1.0	0.5	0.7	0.8	0.6	0.5	0.6	0.6	0.5	0.8
KW	bolus)	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5
AD		0.5	0.5	0.6	0.5	0.5	0.5	0.5	0.5	0.5	0.5	1.8

Appendix 5 continued overleaf.

Appendix 5 (cont.)

Subject	Study number	Serum GH concentration (mU/L)										
		t = -30	t = -15	t = 0	t = 15	t = 30	t = 45	t = 60	t = 75	t = 90	t = 105	t = 120
RR	10	0.5	0.5	0.5	5.3	26.3	30.7	22.2	15.2	7.8	4.9	2.9
MH	(SS50	0.8	1.1	1.0	16.2	25.3	18.0	13.4	8.0	4.4	2.0	2.0
PL	infusion;	0.8	1.0	0.8	3.5	6.9	7.7	7.4	4.1	2.1	1.3	0.9
RH	hexarelin	0.5	0.6	0.5	18.4	52.0	55.5	33.8	22.0	11.0	6.6	5.4
KW	bolus)	0.3	0.5	0.9	2.2	9.3	8.2	14.1	9.0	1.8	1.4	0.9
AD		0.9	0.6	0.7	1.6	2.8	1.1	1.0	0.9	0.5	0.5	0.5
RR	11	0.5	0.5	0.5	12.3	12.6	14.9	8.6	4.0	2.1	1.3	1.0
MH	(SS50	2.4	1.5	1.1	1.6	3.1	3.2	2.0	1.7	1.4	1.3	0.8
PL	infusion;	6.5	10.6	7.7	32.5	23.6	10.9	5.4	3.7	2.3	1.3	1.0
RH	GHRH	0.5	0.5	0.5	0.6	2.2	2.1	2.3	1.6	1.2	1.0	0.5
KW	bolus)	0.5	0.7	0.5	0.5	0.5	0.5	0.6	0.5	0.8	0.5	0.6
AD		0.5	0.6	0.5	1.1	1.4	1.6	2.0	1.5	1.1	0.9	0.7
RR	12	0.5	0.5	0.5	53.7	73.5	73.8	60.4	54.5	43.0	51.1	49.0
MH	(SS50	0.9	0.8	1.0	71.2	59.8	50.5	38.0	27.5	16.6	9.8	6.7
PL	infusion;	1.2	1.2	0.8	35.7	37.5	30.3	17.6	11.6	7.8	3.7	2.6
RH	hex + GHRH	0.8	0.6	0.5	64.6	57.1	55.5	47.1	38.9	30.5	27.6	21.2
KW	bolus)	0.9	0.7	0.6	23.6	25.3	15.9	14.2	8.4	6.8	5.4	4.1
AD		0.5	0.5	0.5	56.3	38.1	36.8	32.1	19.9	16.8	8.5	5.0

SS20: somatostatin infusion at 20 micrograms/m^2/hr
SS50: somatostatin infusion at 50 micrograms/m^2/hr
hex: hexarelin

Appendix 6. Data (serum GH concentration at each sampling point) for the effect of somatostatin withdrawal on the GH response to hexarelin, GHRH-(1-29)-NH_2 or both. Study design is shown in Figure 9a.

Subject	Study number	Serum GH concentration (mU/L)																						
		t=-30	t=-15	t=0	t=15	t=30	t=45	t=60	t=75	t=90	t=105	t=120	t=125	t=130	t=135	t=140	t=145	t=150	t=155	t=160	t=165	t=170	t=175	t=180
MH	1	0.9	0.8	0.7	0.8	0.8	0.7	0.5	0.7	0.7	0.8	1.1	6.0	10.3	16.3	17.6	21.3	29.1	30.7	32.1	31.5	28.2	29.8	28.3
RT	(saline	1.8	2.1	1.2	0.9	0.8	0.6	0.5	0.5	0.7	0.7	0.5	0.5	0.5	0.7	0.8	1.2	2.3	6.1	10.1	12.5	14.9	21.0	23.4
KW	withdrawal;	0.5	0.5	0.5	0.5	0.5	0.5	1.2	0.8	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5
PJ	saline	0.5	0.5	0.5	0.6	0.5	0.5	0.5	0.5	0.5	1.8	4.2	4.4	3.1	2.9	2.2	1.9	1.4	1.3	1.3	0.8	0.7	0.7	0.6
RR	bolus)	1.3	4.2	12.3	18.1	26.7	31.6	36.2	25.2	10.9	7.5	5.3	4.2	3.3	2.8	2.1	1.9	1.7	1.6	1.5	1.4	1.3	1.4	1.3
DS		0.8	0.7	0.8	0.6	0.8	0.7	0.7	0.7	0.7	1.1	2.1	4.3	6.3	6.3	5.6	3.9	2.6	2.3	2.1	1.5	1.4	1.1	1.2
MH	2	0.7	0.7	0.8	0.6	0.5	0.5	0.5	0.6	0.8	1.0	3.7	36.6	75.1	100.5	117.1	129.2	135.0	114.6	112.2	95.9	121.3	87.3	87.0
RT	(saline	0.5	0.5	0.5	0.7	6.3	16.6	11.1	7.9	5.7	4.6	4.1	31.2	88.4	76.5	106.0	116.4	86.6	119.1	113.5	87.6	49.6	60.5	38.1
KW	withdrawal;	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	1.9	7.7	16.0	23.8	41.9	55.4	54.2	57.4	50.6	47.3	46.2	44.1
PJ	hexarelin	0.5	0.6	1.1	0.5	0.5	0.5	1.4	0.8	0.5	0.5	0.5	2.0	23.8	35.1	62.2	85.3	113.1	81.5	120.1	89.6	95.2	91.0	83.5
RR	bolus)	0.6	0.6	0.6	0.6	0.6	0.5	0.5	0.5	0.7	1.2	8.3	21.3	57.5	91.2	120.4	77.2	157.6	126.0	142.6	124.8	107.0	101.9	75.0
DS		0.8	0.9	0.7	0.8	0.9	1.4	8.4	11.4	14.9	10.3	2.4	29.4	54.4	94.4	140.4	130.2	96.6	92.2	85.9	88.1	81.5	69.2	65.0
MH	3	2.0	1.4	0.9	0.8	0.7	0.8	0.5	0.6	0.5	0.5	0.6	9.3	25.4	50.2	64.0	78.4	82.1	72.4	72.4	73.6	73.8	77.8	73.8
RT	(saline	0.6	0.7	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.6	0.5	2.6	11.5	17.1	26.3	31.1	37.2	41.8	52.4	47.7	53.4	50.3	46.7
KW	withdrawal;	0.6	0.7	0.5	0.5	0.5	0.5	0.6	0.5	0.7	0.5	0.5	14.3	28.9	35.0	32.8	30.7	30.5	29.6	32.3	28.2	33.0	28.2	26.7
PJ	GHRH	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.9	1.0	1.1	6.8	25.4	60.3	64.3	61.9	58.3	52.0	49.3	40.6	37.3	29.8	24.0	17.7
RR	bolus)	0.6	0.7	0.6	0.5	0.6	0.5	0.8	1.2	1.0	0.7	1.2	22.9	54.3	83.1	99.8	87.1	109.4	89.8	80.6	82.0	67.9	59.7	49.3
DS		6.1	5.2	2.2	1.2	0.7	0.5	0.5	0.8	0.5	0.5	0.5	1.2	5.1	7.5	10.5	12.5	12.1	11.4	11.0	12.2	17.2	22.1	26.7
MH	4	1.5	1.1	0.6	0.7	0.7	0.7	0.6	0.5	0.5	0.6	1.6	116.5	92.0	141.0	147.0	153.0	142.0	201.0	187.0	181.0	186.0	137.0	144.0
RT	(saline	0.5	0.5	0.5	0.5	0.5	0.5	0.9	6.6	19.4	33.8	26.9	151.0	173.0	254.0	246.0	246.0	234.0	219.0	154.0	208.0	175.0	155.0	143.0
KW	withdrawal;	0.9	0.8	0.5	0.6	0.5	0.6	0.5	0.5	0.6	0.5	0.5	34.4	48.9	57.5	58.1	58.1	55.4	61.3	52.3	54.0	47.9	41.2	45.1
PJ	hex +	4.1	5.1	10.4	12.8	7.2	2.7	1.9	1.0	0.8	0.9	0.5	68.8	103.5	105.0	155.0	146.0	179.4	195.2	175.8	206.2	203.8	146.0	179.6
RR	GHRH	0.6	0.5	0.7	0.6	0.6	0.7	0.6	0.7	0.7	1.0	2.2	84.7	160.8	185.6	224.0	218.8	226.8	234.4	271.6	251.2	262.8	218.4	252.0
DS	bolus)	0.5	0.6	0.7	0.9	0.5	0.9	1.3	0.9	0.9	0.7	0.5	130.0	165.2	227.6	217.2	267.6	214.4	236.0	206.8	222.8	232.0	191.0	173.4

Appendix 6 continued overleaf.

Appendix 6 (cont.)

Subject	Study	Serum GH concentration (mU/L)																						
	number	t=-30	t=-15	t=0	t=15	t=30	t=45	t=60	t=75	t=90	t=105	t=120	t=125	t=130	t=135	t=140	t=145	t=150	t=155	t=160	t=165	t=170	t=175	t=180
MH	5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.6	0.8	0.9	1.4	1.4	2.0	3.4
RT	(SS20	11.3	6.2	2.8	1.7	1.1	0.5	0.6	0.5	0.5	0.5	0.5	0.5	1.1	1.6	7.9	16.6	26.6	44.3	56.0	61.2	60.4	69.4	62.4
KW	withdrawal;	0.7	0.6	0.6	0.5	0.5	0.5	0.5	0.5	0.5	0.6	0.5	0.5	0.5	0.5	0.9	0.5	0.9	0.5	0.6	0.5	0.5	0.5	0.6
PJ	saline	2.3	1.7	1.1	0.7	0.7	0.7	0.7	0.7	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5
RR	bolus)	0.7	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.6	0.5	0.5	0.5	0.6	0.5	0.8	1.0	1.2	1.3	2.1	2.6	3.4	4.0	4.5
DS		0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5
MH	6	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	1.9	22.4	48.6	92.5	116.8	165.0	131.6	102.0	122.8	101.6	123.8	110.2
RT	(SS20	1.2	0.6	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	4.1	23.7	39.3	61.0	119.2	87.0	128.8	116.2	121.6	79.2	61.1	38.2
KW	withdrawal;	0.7	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.8	13.8	32.9	54.1	69.3	72.3	77.8	75.3	68.6	60.4	64.6	52.6
PJ	hexarelin	0.8	0.7	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	3.9	15.9	30.8	57.4	78.8	101.9	109.1	121.4	123.7	121.4	117.6	85.6
RR	bolus)	0.9	1.5	0.8	0.8	0.7	0.5	0.5	0.5	0.6	0.5	0.5	1.6	10.3	32.0	56.1	86.9	130.5	140.2	169.2	200.6	136.2	171.2	150.0
DS		0.9	1.1	0.9	1.2	1.7	1.7	1.4	1.2	0.9	1.1	0.7	3.1	25.3	65.2	96.6	146.9	100.1	142.8	149.2	147.6	107.3	140.9	151.1
MH	7	0.5	0.5	0.8	0.5	0.5	0.5	0.5	0.0	0.0	0.0	0.0	0.5	7.5	17.2	23.4	27.4	33.6	40.8	46.3	55.7	53.1	65.0	62.6
RT	(SS20	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.9	9.3	8.7	11.0	11.4	11.4	10.3	10.4	9.7	11.7	10.4	8.8
KW	withdrawal;	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	3.0	8.2	12.0	14.1	15.4	19.8	21.0	19.5	18.3	17.2	16.2
PJ	GHRH	0.5	0.5	0.7	0.5	0.5	0.6	0.5	1.0	2.3	2.9	1.6	33.2	57.5	59.9	66.7	61.1	70.6	68.3	73.7	59.5	56.4	50.3	40.7
RR	bolus)	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	3.1	7.1	11.2	19.3	34.1	42.7	61.2	74.1	74.3	76.6	72.9	67.2
DS		0.6	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	10.8	22.2	33.4	35.7	40.2	38.1	34.9	38.2	40.6	36.9	37.5	34.4
MH	8	0.9	1.0	0.6	0.6	0.6	0.5	0.5	0.5	0.5	0.5	0.5	71.5	120.6	99.0	141.0	151.0	198.0	158.0	186.0	199.0	200.0	199.0	215.0
RT	(SS20	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	73.0	104.0	141.0	119.0	150.0	106.0	152.0	150.0	135.0	150.0	95.0	83.0
KW	withdrawal;	0.5	0.5	0.5	0.5	0.5	0.5	0.6	0.5	0.5	0.5	0.5	29.3	44.0	54.0	60.7	56.6	50.1	62.2	56.3	56.8	54.1	48.8	49.1
PJ	hex +	4.3	7.0	4.0	2.2	1.3	0.9	0.6	0.8	0.5	0.5	0.5	54.3	88.3	96.6	119.7	135.4	103.0	100.4	130.8	115.8	101.6	105.6	125.8
RR	GHRH	0.8	0.9	0.7	0.5	0.5	0.5	0.5	0.5	1.4	0.5	0.5	49.6	105.8	122.0	149.6	194.0	247.0	246.2	278.2	242.2	246.0	245.0	236.0
DS	bolus)	0.5	0.5	0.5	0.5	0.5	0.5	0.5	1.1	0.5	0.5	0.5	76.6	128.8	164.0	186.8	209.6	166.0	196.8	186.0	209.6	210.4	190.0	184.0

SS20: somatostatin infusion at 20 micrograms/m^2/hr
hex: hexarelin

Appendix 7. Serum GH, PRL and cortisol data for the dose-response studies. Study design is shown in Figure 10.

Subject	Study number	GH concentration (mU/L)		PRL concentration (mU/L)		Cortisol concentration (nmol/L)	
		baseline	peak/trough	baseline	peak/trough	baseline	peak/trough
RH	1	0.5	1.9	148	153	299	225
AD	(saline)	0.8	2.8	558	907	238	315
RR		0.8	1.5	96	172	410	286
MH		0.8	1.5	108	148	326	363
DS		1.7	1.9	129	138	283	445
JA		2.4	5.5	107	73	251	84
RH	2	0.5	9.4	78	116	154	167
AD	(hexarelin	0.7	0.6	888	589	518	267
RR	0.125	1.2	27.2	125	178	314	392
MH	mcg/kg)	1.0	6	124	240	132	260
DS		0.5	2.1	199	202	223	224
JA		1.4	2	131	155	474	300
RH	3	0.5	23.6	87	276	151	180
AD	(hexarelin	1.9	14.6	770	897	426	532
RR	0.25	0.5	38.3	59	132	361	244
MH	mcg/kg)	0.5	22	120	202	185	341
DS		0.7	72.9	121	177	407	457
JA		1.4	1.8	181	269	186	100
RH	4	0.5	22.3	110	264	139	168
AD	(hexarelin	0.5	1.9	1118	1167	308	350
RR	0.375	2.7	35.4	168	333	193	97
MH	mcg/kg)	1.7	4.2	142	294	72	229
DS		0.5	27.8	197	274	204	286
JA		0.5	59.9	126	224	384	200
RH	5	0.9	39.2	142	448	130	172
AD	(hexarelin	1.2	109.8	918	1022	421	572
RR	0.50	0.5	162.1	91	330	295	339
MH	mcg/kg)	1.2	51.6	126	460	98	294
DS		1.4	49.3	91	213	243	450
JA		0.8	154.6	162	270	304	336
RH	6	0.5	60.9	138	720	127	386
AD	(hexarelin	1.5	58	937	1091	432	438
RR	0.75	0.8	163.9	83	331	265	343
MH	mcg/kg)	0.5	60.4	127	361	208	424
DS		1.9	148.6	135	287	358	445
JA		1.3	85.5	145	295	388	130
RH	7	0.5	68.7	129	459	247	515
AD	(hexarelin	0.8	114.3	615	970	307	433
RR	1.00	0.7	272.2	192	375	406	448
MH	mcg/kg)	0.5	66.1	110	373	218	282
DS		1.5	103.4	117	257	314	442
JA		0.9	173.6	153	404	310	371

mcg: microgram

Appendix 8. Serum TSH data for the dose-response studies. Study design is shown in Figure 10.

Subject	Study	Serum TSH concentration (mU/L)										
	number	t = -30	t = -15	t = 0	t = 15	t = 30	t = 45	t = 60	t = 75	t = 90	t = 105	t = 120
RH	1	0.6	0.9	0.7	0.7	0.9	0.9	1.1	0.9	0.8	0.8	0.8
AD	(saline)	1.1	1.0	0.9	0.9	1.0	1.1	1.1	0.8	1.1	1.2	1.1
RR		0.9	0.8	0.8	0.9	0.7	0.7	1.0	0.7	0.9	0.7	1.0
MH		1.2	0.9	1.2	0.9	0.9	0.9	0.8	0.6	0.8	0.9	0.9
DS		1.9	1.9	1.5	1.7	1.3	1.8	1.7	1.7	1.5	1.7	1.8
JA		1.4	1.3	1.2	1.2	1.2	1.2	1.2	1.1	1.1	1.0	1.1
RH	2	1.3	1.6	1.4	1.2	1.4	1.4	1.3	1.4	1.4	1.5	1.5
AD	(hexarelin	1.0	0.8	0.8	0.8	0.9	0.9	0.9	0.8	0.8	0.7	0.9
RR	0.125	1.7	2.0	2.0	2.1	2.0	1.9	1.9	1.8	1.7	1.8	1.5
MH	mcg/kg)	0.5	0.3	0.4	0.2	0.4	0.4	0.3	0.6	0.5	0.4	0.4
DS		0.7	0.7	0.9	0.9	0.7	0.8	0.7	0.7	0.8	0.7	0.7
JA		1.8	1.7	1.5	1.4	1.3	1.3	1.2	1.1	1.2	1.2	1.1
RH	3	2.4	2.2	2.2	2.2	2.3	2.6	2.5	2.3	2.3	2.1	2.3
AD	(hexarelin	1.0	1.0	0.9	0.9	1.0	1.1	1.0	1.0	0.9	0.9	0.9
RR	0.25	1.9	1.9	1.8	2.0	1.8	1.8	2.0	1.9	1.8	1.8	1.9
MH	mcg/kg)	2.1	2.0	2.0	1.8	2.0	1.8	1.7	1.7	1.7	1.6	1.6
DS		1.0	1.0	1.0	0.9	0.8	0.9	0.9	0.8	0.7	0.7	0.8
JA		2.4	2.1	2.0	2.4	2.3	2.0	2.0	1.8	2.0	1.5	2.0
RH	4	0.9	0.9	0.9	0.9	1.2	1.1	1.1	1.1	1.0	1.0	1.1
AD	(hexarelin	0.6	0.7	0.4	0.4	0.4	0.3	0.5	0.5	0.4	0.4	0.4
RR	0.375	1.0	0.5	1.1	1.8	1.0	1.1	0.9	1.0	0.8	0.9	1.2
MH	mcg/kg)	0.4	0.8	0.3	0.8	0.3	0.4	0.3	0.7	0.3	0.6	0.6
DS		1.5	1.4	1.4	1.1	1.3	1.2	1.4	1.3	1.4	1.2	1.3
JA		1.0	1.0	1.0	1.0	0.8	0.8	0.9	0.8	0.7	0.8	0.7
RH	5	2.3	2.1	2.0	2.2	2.1	2.1	2.4	2.2	2.0	2.0	1.9
AD	(hexarelin	0.9	1.3	1.1	1.1	1.5	1.2	1.4	1.1	1.1	1.0	0.8
RR	0.50	2.1	2.2	2.0	2.1	2.1	2.1	2.0	1.9	1.6	2.0	1.9
MH	mcg/kg)	2.2	1.9	2.0	1.9	1.9	2.0	1.9	1.8	1.7	1.6	1.4
DS		1.5	1.4	1.7	1.4	1.4	1.5	1.5	1.4	1.2	1.4	1.5
JA		2.1	2.2	2.2	2.1	2.0	1.9	1.8	1.9	1.6	1.7	1.5
RH	6	2.5	2.1	1.9	2.1	2.1	2.2	2.0	2.2	2.1	2.0	1.9
AD	(hexarelin	1.2	1.2	1.2	1.1	1.1	1.0	1.1	1.0	1.1	1.1	1.4
RR	0.75	1.1	0.9	1.0	0.9	0.6	0.9	1.1	1.3	1.1	1.4	0.9
MH	mcg/kg)	0.8	0.8	0.8	0.9	0.9	0.8	0.8	0.8	1.0	0.7	0.7
DS		1.2	1.1	1.1	1.1	1.0	1.0	0.9	1.0	0.9	0.9	0.8
JA		1.3	1.3	1.3	1.2	1.2	1.1	1.0	0.7	0.9	0.8	0.8
RH	7	0.8	1.0	1.0	0.6	0.6	0.8	1.3	0.9	1.0	0.7	1.0
AD	(hexarelin	1.9	1.8	1.6	1.4	1.7	1.5	1.5	1.4	1.6	1.4	1.6
RR	1.00	1.0	1.3	0.9	1.3	0.5	0.6	0.7	0.8	0.8	0.6	0.8
MH	mcg/kg)	2.1	1.9	1.8	1.8	1.9	1.8	1.8	1.6	1.6	1.2	1.2
DS		2.4	2.2	2.3	2.0	1.9	1.3	1.7	1.0	1.9	1.6	1.6
JA		0.9	0.9	0.8	0.5	1.1	0.4	0.5	0.8	0.5	0.6	0.7

mcg: microgram

Appendix 9. Serum insulin data for the dose-response studies. Study design is shown in Figure 10.

Subject	Study number	Serum insulin concentration (iU/L)										
		t = -30	t = -15	t = 0	t = 15	t = 30	t = 45	t = 60	t = 75	t = 90	t = 105	t = 120
RH	1	20.4	11.6	11.0	4.5	8.1	14.9	14.2	8.0	4.2	10.4	10.1
AD	(saline)	8.5	6.1	7.4	5.9	2.1	3.8	4.6	3.9	1.8	4.2	2.6
RR		4.9	3.6	5.8	2.3	4.3	4.6	4.1	4.2	3.8	4.5	5.2
MH		6.0	6.6	6.6	3.8	3.5	5.1	6.9	4.9	6.2	8.2	6.3
DS		6.0	4.8	4.7	5.8	5.6	4.5	4.7	5.7	3.9	3.7	5.0
JA		6.7	6.6	5.6	3.4	4.6	4.8	4.1	5.5	7.2	6.4	9.2
RH	2	10.9	8.4	7.9	2.3	1.8	1.5	4.3	1.5	3.3	3.5	5.5
AD	(hexarelin	2.2	3.7	1.8	1.7	1.8	1.3	1.5	1.5	1.5	1.5	1.8
RR	0.125	1.5	1.5	1.7	4.0	1.5	1.5	1.7	1.5	1.9	1.5	1.5
MH	mcg/kg)	2.0	1.9	1.5	2.0	1.5	2.6	2.0	1.8	2.5	2.8	2.3
DS		3.0	2.2	3.0	2.1	3.0	2.4	3.2	3.9	2.8	2.4	1.8
JA		4.4	3.1	2.9	4.2	3.8	3.2	5.0	3.9	5.5	3.1	3.9
RH	3	12.1	10.8	6.2	6.8	4.6	8.3	5.7	8.4	12.5	11.8	7.9
AD	(hexarelin	2.1	2.9	3.1	1.8	2.4	1.5	3.8	2.4	3.3	2.3	2.1
RR	0.25	17.6	7.7	3.5	1.5	6.2	5.6	8.6	16.8	2.9	3.8	11.2
MH	mcg/kg)	2.0	3.7	4.3	3.5	3.4	1.5	3.0	1.5	1.8	1.9	1.5
DS		3.9	3.5	4.3	4.5	2.8	2.9	3.7	1.1	4.4	2.0	3.4
JA		5.4	5.4	5.5	4.8	4.3	4.6	5.3	4.1	4.3	4.4	5.4
RH	4	3.5	15.9	5.4	4.2	6.6	7.2	2.5	3.1	6.1	10.1	12.1
AD	(hexarelin	7.1	10.6	12.4	6.6	6.4	4.8	4.1	5.4	2.3	3.8	5.7
RR	0.375	1.5	2.2	1.6	1.5	1.5	1.7	1.9	1.5	1.5	1.7	2.7
MH	mcg/kg)	4.6	2.3	2.8	2.3	1.8	2.6	2.3	2.8	3.9	2.5	3.2
DS		3.5	3.3	2.6	2.8	2.4	2.4	2.3	2.9	4.5	3.1	2.7
JA		6.2	6.6	6.2	5.4	3.5	3.2	2.4	3.2	3.2	4.6	2.6
RH	5	8.7	5.9	6.5	4.4	3.6	4.5	4.7	4.3	2.9	3.8	7.3
AD	(hexarelin	2.3	1.5	1.5	3.2	2.9	1.9	2.8	2.4	5.1	4.2	1.5
RR	0.50	6.6	4.1	3.0	3.2	2.4	1.5	3.0	1.8	1.9	3.1	4.3
MH	mcg/kg)	1.6	1.5	1.6	1.5	1.5	1.5	1.6	1.8	2.5	1.6	1.5
DS		3.3	3.3	3.0	4.5	3.7	2.4	1.5	3.5	4.6	3.3	3.1
JA		5.9	5.2	5.6	5.1	4.6	3.8	4.5	3.8	4.4	5.0	5.9
RH	6	9.1	8.5	9.0	6.3	7.3	8.0	7.6	7.3	6.2	9.5	10.2
AD	(hexarelin	2.9	6.1	2.5	2.0	2.3	1.5	2.9	2.0	0.5	1.3	1.7
RR	0.75	3.7	3.3	3.6	1.5	1.9	2.6	2.0	2.3	1.7	1.5	5.9
MH	mcg/kg)	1.5	1.5	1.5	1.5	1.5	1.5	1.5	1.8	1.7	1.6	1.8
DS		2.4	2.2	2.3	1.4	1.9	2.4	2.0	1.5	3.1	2.7	3.3
JA		2.7	3.4	3.9	3.0	2.9	2.6	3.4	2.9	3.4	5.3	4.5
RH	7	7.5	1.5	6.4	1.5	1.5	2.8	6.1	1.5	2.0	4.4	1.5
AD	(hexarelin	3.0	3.7	1.8	1.8	1.6	1.9	1.5	2.1	2.6	2.6	2.5
RR	1.00	7.6	6.2	5.3	5.3	4.9	4.1	4.8	4.8	4.0	4.6	3.5
MH	mcg/kg)	1.4	1.5	1.5	1.5	1.5	1.5	1.5	1.5	1.5	1.5	1.5
DS		5.9	4.1	4.2	5.0	4.3	3.8	4.1	5.1	7.6	5.8	5.0
JA		4.5	2.8	3.6	1.6	2.3	2.0	2.2	1.5	1.7	2.8	3.9

mcg: microgram

Appendix 10. Blood glucose data for the dose-response studies. Study design is shown in Figure 10.

Subject	Study number	Blood glucose concentration (mmol/L)										
		t = -30	t = -15	t = 0	t = 15	t = 30	t = 45	t = 60	t = 75	t = 90	t = 105	t = 120
RH	1	4.4	4.5	4.3	4.4	3.9	4.4	3.8	3.9	3.9	3.7	3.5
AD	(saline)	5.0	4.8	4.7	4.7	4.3	4.2	4.2	4.2	3.9	3.8	3.7
RR		3.9	3.8	3.8	3.7	4.0	4.0	4.1	4.0	4.0	3.9	3.5
MH		4.1	3.9	3.7	3.7	3.6	3.7	3.8	3.7	3.8	3.7	3.6
DS		4.5	4.6	4.4	4.4	4.3	4.3	4.5	4.6	4.4	4.4	4.5
JA		4.6	4.5	4.3	4.3	4.3	4.3	4.2	4.0	4.2	4.0	4.1
RH	2	5.4	5.3	5.8	5.4	5.8	5.8	5.6	5.3	5.8	5.5	5.5
AD	(hexarelin	6.0	6.7	7.2	6.8	6.0	6.4	5.6	5.9	5.7	5.6	5.5
RR	0.125	6.4	5.9	5.7	6.2	5.7	5.6	5.3	5.5	5.1	5.7	5.9
MH	mcg/kg)	3.8	3.6	3.5	3.6	3.6	3.6	3.5	3.4	3.6	3.5	3.5
DS		4.3	4.4	4.3	4.2	4.3	4.2	4.3	4.3	4.3	4.1	3.9
JA		5.6	5.9	6.1	5.8	6.0	6.4	6.6	6.1	6.4	6.0	6.4
RH	3	3.9	3.9	3.8	3.8	3.6	3.8	3.9	3.7	3.3	3.4	3.3
AD	(hexarelin	3.1	3.1	3.2	3.2	3.2	3.3	3.2	3.3	3.5	3.6	3.6
RR	0.25	4.2	2.9	3.3	3.4	4.1	4.2	5.2	4.9	4.5	2.4	3.9
MH	mcg/kg)	3.8	3.8	3.3	3.6	3.5	3.6	3.3	3.2	3.2	3.3	3.3
DS		4.1	4.1	4.0	3.9	3.8	3.7	3.6	3.7	3.6	4.0	3.7
JA		4.0	3.9	3.7	3.9	4.1	3.8	3.8	3.8	3.6	3.7	3.8
RH	4	5.0	5.0	5.4	5.0	5.0	4.8	4.9	4.6	4.4	4.2	4.3
AD	(hexarelin	5.9	5.4	5.7	5.7	5.6	5.1	5.2	5.4	5.2	5.1	5.6
RR	0.375	3.8	3.8	3.6	3.7	3.7	3.4	3.5	3.4	3.5	3.4	3.5
MH	mcg/kg)	3.7	3.6	3.9	3.8	3.8	3.9	3.9	3.9	3.7	3.7	3.7
DS		4.5	4.4	4.3	4.4	4.3	4.2	4.4	4.2	4.4	4.2	4.1
JA		6.3	6.1	6.3	6.1	6.3	5.6	5.6	5.7	6.0	5.7	5.7
RH	5	4.1	4.0	3.7	4.0	3.9	3.7	3.7	3.6	3.6	3.6	3.6
AD	(hexarelin	4.4	4.2	4.1	4.1	4.2	3.9	3.8	4.2	3.8	3.9	4.0
RR	0.50	4.2	3.6	3.4	3.5	3.5	3.7	3.3	3.4	3.7	3.5	3.6
MH	mcg/kg)	3.9	3.9	3.7	3.7	3.6	3.6	3.3	3.2	3.3	3.5	3.4
DS		3.8	4.0	3.7	4.1	3.7	3.8	3.8	3.8	3.9	3.8	3.9
JA		3.8	3.7	3.6	3.6	3.5	3.4	3.6	3.4	3.5	3.6	3.6
RH	6	3.7	3.8	3.7	3.6	3.6	3.5	3.1	3.3	3.1	3.1	3.4
AD	(hexarelin	4.3	4.0	3.6	4.2	4.0	3.8	3.6	3.7	3.7	3.8	3.5
RR	0.75	3.5	3.5	3.9	4.0	3.9	3.7	4.0	4.0	3.9	3.9	3.8
MH	mcg/kg)	3.6	3.9	3.8	3.6	3.8	3.4	3.5	3.6	3.4	3.3	3.5
DS		3.7	3.3	3.4	3.4	2.9	3.4	3.4	3.5	3.2	3.4	3.3
JA		4.1	4.2	3.8	3.8	4.1	3.8	3.8	3.9	4.0	4.0	4.0
RH	7	4.1	4.2	3.7	4.0	3.9	3.7	3.5	3.5	3.5	3.7	3.7
AD	(hexarelin	4.4	4.2	3.9	4.2	4.0	3.9	3.7	3.8	3.8	3.6	3.7
RR	1.00	4.0	3.2	3.6	3.2	3.6	3.3	3.5	3.6	3.6	3.7	3.7
MH	mcg/kg)	3.7	3.5	3.3	3.2	3.1	3.3	3.3	3.4	3.3	3.6	3.4
DS		3.7	3.8	3.5	3.7	3.4	3.6	3.3	3.3	3.3	3.2	3.3
JA		4.2	3.9	4.2	4.1	4.0	4.0	4.0	4.0	4.0	3.8	4.2

mcg: microgram

Appendix 11. Serum GH, PRL, cortisol, TSH and insulin and blood glucose data for the GHRH-(1-29)-NH_2 and the combined low dose hexarelin plus GHRH-(1-29)-NH_2 studies. Study design is shown in Figure 10.

Subject	Study	GH conc. (mU/L)		PRL conc. (mU/L)		Cortisol conc. (nmol/L)		TSH conc. (mU/L)		Insulin conc. (iU/L)		Glucose conc. (mmol/L)	
	number	baseline	peak/trough	baseline	peak/trough	baseline	peak/trough	baseline	peak/trough	baseline	peak/trough	baseline	peak/trough
RH	8	0.5	12.8	71	178	277	137	0.7	1.7	17.6	7.1	4.3	4.8
AD	(GHRH	1.3	33.9	939	942	366	406	0.4	0.7	3.8	1.5	3.8	4.2
RR	1.00	1.3	58.9	83	191	386	217	1.7	2.1	8.5	5.5	4.3	5.0
MH	mcg/kg)	0.7	65.0	77	114	267	102	1.4	1.5	6.1	7.5	4.0	3.5
DS		1.8	34.9	95	137	200	145	1.7	1.8	6.2	3.7	4.3	3.6
JA		1.8	49.6	92	146	233	234	1.3	1.5	3.7	6.5	4.0	3.7
RH	9	0.6	37.5	213	477	213	93	1.6	2.0	5.0	1.5	3.7	3.3
AD	(hexarelin	xxx	xxx	xxx	xxx	xxx	xxx	xxx	xxx	xxx	xxx	xxx	xxx
RR	0.125	xxx	xxx	xxx	xxx	xxx	xxx	xxx	xxx	xxx	xxx	xxx	xxx
MH	mcg/kg	0.5	129.8	76	182	113	213	0.9	1.1	6.9	2.1	3.9	3.3
DS	+ GHRH	0.5	100.7	187	134	200	155	1.9	1.4	1.5	1.5	4.8	3.9
JA	1.00 mcg/kg)	0.5	195.5	73	108	288	90	1.3	1.0	1.5	1.7	5.3	5.3

mcg: microgram
conc.: concentration

REFERENCES

Achermann J C, Hindmarsh P C, Brook C G D 1996 Combining continuous growth hormone releasing hormone (GHRH) with intermittent somatostatin (SS) generates repeatable growth hormone (GH) responses in man. Hormone Research 46(suppl 2):7(abstract).

Akman M S, Girard M, O'Brien L F, Ho A K, Chik C L 1993 Mechanism of action of a second generation growth hormone-releasing peptide (Ala-His-D-ßNal-Ala-Trp-D-Phe-Lys-NH_2) in rat anterior pituitary cells. Endocrinology 132:1286-1291.

Albertsson-Wikland K, Rosberg S 1988 Analysis of 24-hour growth hormone profiles in children: relation to growth. J Clin Endocrinol Metab 67:493-500.

Alster D K, Bowers C Y, Jaffe C A, Ho P J, Barkan A L 1993 The growth hormone (GH) response to GH-releasing peptide (His-DTrp-Ala-Trp-DPhe-Lys-NH_2), GH-releasing hormone, and thyrotropin-releasing hormone in acromegaly. J Clin Endocrinol Metab 77:842-845.

Arosio M, Losa M, Bazzoni N, Bohicchio D, Palmieri E, Nava C, Faglia G 1990 Effects of propranolol on GH responsiveness to repeated GH-releasing hormone stimulations in normal subjects. Acta Endocrinol 122:735-739.

Arvat E, Ghigo E, Nicolosi M, Boffano G M, Bellone J, Yin-Zhang W, Mazza E, Camanni F 1992 Galanin reinstates the growth hormone response to repeated growth hormone-releasing administration in man. Clin Endocrinol 36:347-350.

Arvat E, Gianotti L, Grottoli S, Imbimbo B P, Lenaerts V, Deghenghi R, Camanni F, Ghigo E 1994 Arginine and growth hormone-releasing hormone restore the blunted growth hormone-releasing activity of hexarelin in elderly subjects. J Clin Endocrinol Metab 79:1440-1443.

Arvat E, Gianotti L, Di Vito L, Imbimbo B P, Lenaerts V, Deghenghi R, Camanni F, Ghigo E 1995 Modulation of growth hormone-releasing activity of hexarelin in man. Neuroendocrinology 61:51-56.

Arvat E, Gianotti L, Ramunni J, Di Vito L, Deghenghi R, Camanni G, Ghigo E 1996 Influence of beta-adrenergic agonists and antagonists on the GH-releasing effect of hexarelin in man. J Endocrinol Invest 19:25-29.

Badger T M, Millard W J, McCormick G F, Bowers C Y, Martin J B 1984 The effects of growth hormone (GH)-releasing peptides on GH secretion in perifused pituitary cells of adult male rats. Endocrinology 115:1432-1438.

Barinaga M, Bilezikjian L M, Vale W W, Rosenfeld M G, Evans R M 1985 Independent effects of growth hormone releasing factor on growth hormone release and gene transcription. Nature 314:279-281.

Becker K, Stegenga S, Conway S 1995 Role of insulin-like growth factor-1 in

regulating growth hormone release and feedback in the male rat. Neuroendocrinology 61:573-583.

Bell J P, Donald R A, Espiner E A 1970 Pituitary response to insulin-induced hypoglycaemia in obese subjects before and after fasting. J Clin Endocrinol Metab 31:546-551.

Bellone J, Ghizzoni L, Aimaretti G, Volta C, Boghen M F, Bernasconi S, Ghigo E 1995a Growth hormone-releasing effect of oral growth hormone-releasing peptide (GHRP-6) administration in children with short stature. Eur J Endocrinol 133:425-429.

Bellone J, Aimaretti G, Bartolotta E, Benso L, Imbimbo B P, Lenaerts V, Deghenghi R, Camanni F, Ghigo E 1995b Growth hormone-releasing activity of hexarelin, a new synthetic hexapeptide, before and during puberty. J Clin Endocrinol Metab 80:1090-1094.

Bercu B B, Yang S-W, Masuda R, Walker R F 1992 Role of selected endogenous peptides in growth hormone-releasing hexapeptide activity: analysis of growth hormone-releasing hormone, thyroid hormone-releasing, and gonadotrophin-releasing hormone. Endocrinology 130:2579-2586.

Bercu B B, Walker R F 1996 Evaluation of pituitary function in children using growth hormone secretagogues. J Pediatr Endocrinol Metab 9(suppl 3):325-332.

Berelowitz M, Szabo M, Frohman L A, Firestone S L, Chu L, Hintz R L 1981 Somatomedin-C mediates growth hormone negative feedback by effects on both the hypothalamus and the pituitary. Science 212:1279-1281.

Berridge M J, Irvine R F 1989 Inositol phosphates and cell signalling. Nature 341:197-205.

Bowers C Y, Alster D K, Frentz J M 1992 The growth hormone-releasing activity of a synthetic hexapeptide in normal men and short statured children after oral administration. J Clin Endocrinol Metab 74:292-298.

Billestrup N, Mitchell R T, Vale W 1987 Growth hormone releasing factor induces c-fos expression in cultured primary pituitary cells. Mol Endocrinol 1:300-305.

Bitar K G, Bowers C Y, Coy D H 1991 Effects of substance P/bombesin antagonists on the release of growth hormone by GHRP and GHRH. Biochem Biophys Res Commun 180:156-161.

Blake A D, Smith R G 1991 Desensitization studies using perifused rat pituitary cells show that growth hormone-releasing hormone and His-D-Trp-Ala-Trp-D-Phe-Lys-NH_2 stimulate growth hormone release through distinct receptor sites. J Endocrinol 129:11-19.

Bowers C Y 1993a GH releasing peptides - structure and kinetics. J Pediatr

Endocrinol 6:21-31.

Bowers C Y 1993b A new dimension on the induced release of growth hormone in obese subjects [editorial]. J Clin Endocrinol Metab 76:817-818.

Bowers C Y 1994 On a peptidomimetic growth hormone releasing peptide [editorial]. J Clin Endocrinol Metab 79:940-942.

Bowers C Y, Chang J K, Momany F A, Folkers K 1977a Effects of the enkephalins and enkephalin analogs on release of pituitary hormones *in vitro*. Proceedings of the 6th International Conference on Endocrinology, Amsterdam, 1977. 287 (abstract).

Bowers C Y, Chang J K, Fong T T W 1977b A synthetic pentapeptide which specifically releases GH *in vitro*. Proceedings of the 59th Annual Meeting of The American Endocrine Society, 1977. 232 (abstract).

Bowers C Y, Momany F A, Reynolds G A, Chang D, Hong A, Chang K 1980 Structure-activity relationships of a synthetic pentapeptide that specifically releases growth hormone *in vitro*. Endocrinology 106:663-667.

Bowers C Y, Momany F A, Reynolds G A, Hong A 1984 On the *in vitro* and *in vivo* activity of a new synthetic hexapeptide that acts on the pituitary to specifically release growth hormone. Endocrinology 114:1537-1545.

Bowers C Y, Reynolds G A, Durham D, Barrera C M, Pezzoli S S, Thorner M O 1990 Growth hormone (GH)-releasing peptide stimulates GH release in normal men and acts synergistically with GH-releasing hormone. J Clin Endocrinol Metab 70:975-982.

Bowers C Y, Reynolds G A, Barrera C M 1991a A second generation GH releasing heptapeptide. Proceedings of the 73rd Annual Meeting of The American Endocrine Society, Washington, DC, 1991. 422 (abstract).

Bowers C Y, Sartor A O, Reynolds G A, Badger T M 1991b On the actions of the growth hormone-releasing hexapeptide, GHRP. Endocrinology 128:2027-2035.

Bowers C Y, Veeraragavan K, Sethumadhavan K 1994 Atypical growth hormone releasing peptides. In: Bercu BB, Walker RF (eds) Growth Hormone II: Basic and Clinical Aspects. Serono Symposia, New York, p. 203-222.

Brain C E, Hindmarsh P C, Brook C G D, Matthews D R 1988 Continuous subcutaneous growth hormone releasing factor analogue augments growth hormone secretion in normal male subjects with no desensitization of the somatotroph . Clin Endocrinol 28:543-549.

Brain C E, Thakrar D N, Hindmarsh P C, Brook C G D 1993 Physiological levels of growth hormone fail to suppress growth hormone releasing hormone (1-29) NH_2-stimulated growth hormone secretion in man. J Endocrinol Invest

16:15-20.

Bresson-Bepoldin L, Dufy-Barbe L 1994 GHRP-6 induces a biphasic calcium response in rat pituitary somatotroph. Cell Calcium 15:247-258.

Bresson-Bepoldin L, Odessa M F, Dufy-Barbe L 1995 GHRP-6 stimulates calcium increase and growth hormone release in human somatotrophs *in vitro*. Endocrinology 2:793-803.

Burgess J A, Smith B R, Merimee T J 1966 Growth hormone in thyrotoxicosis: effect of insulin-induced hypoglycaemia. J Clin Endocrinol Metab 26:1257-1260.

Burman P, Broman J E, Hetta J, Wiklund I, Erfurth E M, Hagg E, Karlsson F A 1995 Quality of life in adults with growth hormone (GH) deficiency: response to treatment with recombinant human GH in a placebo-controlled 21 month trial. J Clin Endocrinol Metab 80:3585-3590.

Cappa M, Setzu S, Bernardini S, Carta D, Federici G, Grossi A, Loche S 1995 Exogenous growth hormone administration does not inhibit the growth hormone response to hexarelin in normal men. J Endocrinol Invest 18:762-766.

Casanueva F F 1992 Physiology of growth hormone secretion and action. Endocrinol Metab Clin North Am 21:483-517.

Casanueva F F, Bett R, Cella S G, Muller E E, Mantegazza P 1983 Effects of agonists and antagonists of cholinergic neurotransmission on growth hormone release in the dog. Acta Endocrinol 103:15-20.

Casanueva F F, Burguera B, Muruais C, Dieguez C 1990 Acute administration of corticoids: a new and peculiar stimulus of growth hormone secretion in man. J Clin Endocrinol Metab 70:234-237.

Chapman I M, Bach M A, Cauter E V, Farmer M, Krupa D, Taylor A M, Schilling L M, Cole K Y, Skiles E H, Pezzoli S S, Hartman M L, Veldhuis J D, Gormley G J, Thorner M O 1996 Stimulation of the growth hormone (GH)-insulin-like growth factor-1 axis by daily oral administration of a GH secretagogue (MK-0677) in healthy elderly subjects. J Clin Endocrinol Metab 81:4249-4257.

Chen C, Clarke I J 1992 Ion channels in the regulation of growth hormone secretion from somatotrophs by somatostatin. Growth Regul 2:167-174.

Chen C, Clarke I J 1995 Effects of growth hormone-releasing peptide-2 (GHRP-2) on membrane Ca^{2+} permeability in cultured ovine somatotrophs. J Neuroendocrinol 7:179-186.

Cheng K, Chan W W S, Barreto A, Convey E M, Smith R G 1989 The synergistic effects of His-D-Trp-Ala-Trp-D-Phe-Lys-NH_2 on growth hormone (GH)-releasing factor stimulated GH release and intracellular adenosine 3', 5'-monophosphate accumulation in rat primary pituitary cell culture. Endocrinology

124:2791-2798.

Cheng K, Chan W W S, Butler B, Barreto A, Smith R G 1991 Evidence for a role of protein kinase-C in His-D-Trp-Ala-Trp-D-Phe-Lys-NH_2-induced growth hormone release from rat primary pituitary cells. Endocrinology 129:3337-3342.

Cheng K, Chan W W S, Butler B, Wei L, Schoen W R, Wyvratt M J, Fisher M H, Smith R G 1993 Stimulation of growth hormone release from rat pituitary cells by L-692,429, a novel non-peptidyl GH secretagogue. Endocrinology 132:2729-2731.

Chihara K, Minamitami N, Kaji H, Arimura A, Fujita T 1981 Intraventricular injected growth hormone stimulates somatostatin release into rat hypophyseal portal blood. Endocrinology 109:2279-2281.

Chomczynski P, Downs T R, Frohman L A 1988 Feedback regulation of growth hormone (GH)-releasing hormone gene expression by GH in rat hypothalamus. Mol Endocrinol 2:236-241.

Chretien M, Benjannet S, Dragon N, Seidah N G, Lis M 1976 Isolation of peptides with opiate activity from sheep and human pituitaries: relationship to beta-lipotropin. Biochem Biophys Res Commun 72:472-478.

Christiansen J S, Jorgensen J O L, Thuesen L 1996 Cardiovascular aspects of growth hormone replacement therapy in adults. Endocrinol Metab 3(suppl. A):19-22.

Ciccarelli E, Grottoli S, Razzore P, Gianotti L, Arvat E, Camanni G, Ghigo E 1996 Hexarelin, a synthetic growth hormone releasing peptide, stimulates prolactin secretion in acromegalic but not in hyperprolactinaemic patients. Clin Endocrinol 44:67-71.

Clark R G, Carlsson L M S, Rafferty B, Robinson I C A F 1988 The rebound release of growth hormone (GH) following somatostatin infusion in rats involves hypothalamic GH-releasing factor release. J Endocrinol 119:397-404.

Clark R G, Carlsson L M S, Trojnar J, Robinson I C A F 1989 The effects of a growth hormone-releasing peptide and growth hormone-releasing factor in conscious and anaesthetised rats. J Neuroendocrinol 1:249-55.

Codd E E, Yellin T, Walker R F 1988 Binding of growth hormone-releasing hormones and enkephalin-derived growth hormone-releasing peptides to mu and delta opioid receptors in forebrain of rat. Neuropharmacology 27:1019-1025.

Codd E E, Shu A Y L, Walker R F 1989 Binding of a growth hormone releasing hexapeptide to specific hypothalamic and pituitary binding sites. Neuropharmacology 28:1139-1144.

Codd E E, Aloyo V J, Walker R F 1990 A non-opioid pattern characterizes

inhibition of growth hormone releasing peptide binding by dynorphin-related peptides. Neuropeptides 15:133-137.

Conley L K, Teik J A, Deghenghi R, Imbimbo B P, Giustina A, Locatelli V, Wehrenberg W B 1995 Mechanism of action of hexarelin and GHRP-6: analysis of the involvement of GHRH and somatostatin in the rat. Neuroendocrinology 61:44-50.

Copeland K C, Underwood L E, Van Wyk J J 1980 Induction of immunoreactive somatomedin C in human serum by growth hormone: dose-response relationships and effect on chromatographic profile. J Clin Endocrinol Metab 50:690-697.

Copinschi G, Van Onderbergen A, Caufriez A, Mendel C M, L'Hermite-Baleriaux M, Leproult R, Bolognese J A, De Lepeleire I, Thorner M O, Van Cauter E 1996 Effects of 7-day treatment with the orally active growth hormone (GH) secretagogue, MK-677, on insulin-like growth factor (IGF-1) levels, 24-GH and cortisol profiles, and sleep in normal young men. Proceedings of the 10th International Congress of Endocrinology, San Francisco, 1996. 769 (abstract P3-57).

Cordido F, Dieguez C, Casanueva F F 1989 Cholinergic receptor activation by pyridostigmine restores GH responsivenss to GHRH in obese subjects. J Clin Endocrinol Metab 68:290-293.

Cordido F, Dieguez C, Casanueva F F 1990 Effect of central cholinergic enhancement by pyridostigmine on the GH secretion elicited by clonidine, arginine, or hypoglycaemia in normal and obese subjects. J Clin Endocrinol Metab 70:1361-1370.

Cordido F, Peñalva A, Dieguez C, Casanueva F F 1993 Massive growth hormone discharge in obese subjects after the combined administration of GH-releasing hormone and GHRP-6: evidence for a marked somatotroph secretory capability in obesity. J Clin Endocrinol Metab 76:819-822.

Cordido F, Peñalva A, Peino R, Casanueva F F, Dieguez C 1995 Effect of combined administration of growth hormone (GH)-releasing hormone, GH-releasing peptide-6, and pyridostigmine in normal and obese subjects. Metabolism 44:745-748.

Corpas E, Harman S M, Blackman S 1993 Human growth hormone and human aging. Endocr Rev 14:20-39.

Dattani M T, Pringle P J, Hindmarsh P C, Brook C G D 1992 What is a normal stimulated growth hormone concentration? J Endocrinol 133:447-450.

DeBell W K, Pezzoli S S, Thorner M O 1991 Growth hormone (GH) secretion during continuous infusion of GH-releasing peptide: partial response attenuation. J Clin Endocrinol Metab 72:1312-1316.

Deghenghi R, Cananzi M M, Battisti C, Locatelli V, Muller E E 1992 Hexarelin (EP23905) - a superactive growth hormone releasing peptide. J Endocrinol Invest 15(suppl 4):45(abstract).

Deghenghi R, Cananzi M M, Torsello A, Battisti C, Muller E E 1994 GH-releasing activity of hexarelin, a new growth hormone releasing peptide, in infant and adult rats. Life Sci 54:1321-1328.

Deghenghi R, Boutignon F, Luoni M, Grilli R, Guidi M, Locatelli V 1995 Small peptides as potent releasers of growth hormone. J Pediatr Endocrinol Metab 8:311-313.

Deghenghi R, Boutignon F, Luoni M, Grilli R, Guidi M, Locatelli V 1996 Characterization of the activity of new growth hormone secretagogues in the infant rat. Proceedings of the 10th International Congress of Endocrinology, San Francisco, 1996. 280 (abstract P1-581).

Demura R, Demura H, Nunokawa T, Bab H, Miura K 1972 Responses of plasma ACTH, GH, LH and 11-hydroxycorticosteroids to various stimuli in patients with Cushing's syndrome. J Clin Endocrinol Metab 34:852-859.

Devesa J, Lima L, Lois N, Fraga C, Lechuga M J, Arce V, Tresguerres J A F 1989 Reasons for the variability in growth hormone (GH) responses to GHRH challenge: the endogenous hypothalamic-somatotroph rhythm. Clin Endocrinol 30:367-377.

Dickson S L, Leng G, Robinson I C A F 1993 Systemic administration of growth hormone-releasing peptide activates hypothalamic arcuate neurones. Neuroscience 53:303-306.

Dickson S L, Leng G, Dyball R E J, Smith R G 1995a Central actions of peptide and non-peptide growth hormone secretagogues in the rat. Neuroendocrinology 61:36-43.

Dickson S L, Doutrelant-Viltart O, Leng G 1995b GH-deficient *dw/dw* rats and *lit/lit* mice show increased Fos expression in the hypothalamic arcuate nucleus following systemic injection of GH-releasing peptide-6. J Endocrinol 146:519-526.

Dickson S L, Doutrelant-Viltart O, Leng G 1997 Sandostatin attenuation of the GHRP-6-induced fos protein expression in the arcuate nucleus. Endocrinol Metab 4(suppl A):33(abstract).

Dieguez C, Page M D, Scanlon M F 1988 Growth hormone neuroregulation and its alterations in disease states. Clin Endocrinol 28:109-143.

Edwards C A, Dieguez C, Scanlon M F 1989 Effects of hypothyroidism, tri-iodothyronine and glucocorticoids on growth hormone responses to growth hormone-releasing hormone and His-D-Trp-Ala-Trp-D-Phe-Lys-NH_2. J

Endocrinol 121:31-36.

Elias K A, Ingle G S, Butnier J P, Hammonds R G, McDowell R S, Rowson T E, Somers T C, Stanley M S, Cronin M J 1995 *In vitro* characterisation of four novel classes of growth hormone releasing peptide. Endocrinology 136:5694-5699.

Epelbaum J, Moyse E, Tannenbaum G S, Kordon C, Beaudet A 1989 Combined autoradiographic and immunohistochemical evidence for an association of somatostatin binding sites with growth hormone-releasing factor-containing nerve cell bodies in the rat arcuate nucleus. J Neuroendocrinol 1:109-115.

Fairhall K M, Mynett A, Robinson I C A F 1995 Central effects of growth hormone-releasing hexapeptide (GHRP-6) on growth hormone release are inhibited by central somatostatin action. J Endocrinol 144:555-560.

Finkelstein J W, Roffwarg H P, Boyar R M, Kream J, Hellman L 1972 Age-related change in the twenty-four-hour spontaneous secretion of growth hormone. J Clin Endocrinol Metab 35:665-70.

Fletcher T P, Thomas G B, Willoughby J O, Clarke I J 1994 Constitutive growth hormone secretion in sheep after hypothalamopituitary disconnection and the direct *in vivo* effect of growth hormone releasing peptide 6. Neuroendocrinology 60:76-86.

Frantz A G, Rabkin M T 1964 Human growth hormone. Clinical measurement, response to hypoglycaemia and suppression by corticosteroids. N Engl J Med 271:1375-1381.

Frenkel J, Silbergeld A, Deghenghi R, Laron Z 1995 Short term effect of intranasal administration of hexarelin-a synthetic growth hormone-releasing peptide. Preliminary communication. J Pediatr Endocrinol Metab 8:43-45.

Frieboes R-M, Murck H, Maier P, Schier T, Holsboer F, Steiger A 1995 Growth hormone-releasing peptide-6 stimulates sleep, growth hormone, ACTH and cortisol in normal man. Neuroendocrinology 61:584-589.

Frohman L A, Jansson J-O 1986 Growth hormone-releasing hormone. Endocr Rev 7:223-253.

Frohman L A, Downs T R, Chomczynski P 1992 Regulation of growth hormone secretion. Front Neuroendocrinol 13:344-405.

Gelato M C, Pescovitz O H, Cassorla F, Loriaux D L, Merriam G R 1984 Dose-response relationship for the effects of growth hormone-releasing factor-(1-44)-NH_2 in young adult men and women. J Clin Endocrinol Metab 59:197-201.

Gertz B J, Barrett J S, Eisenhandler R, Krupa D A, Wittreich J M, Seibold J R,

Schneider S H 1993 Growth hormone response in man to L-692,429, a novel nonpeptide mimic of growth hormone-releasing peptide-6. J Clin Endocrinol Metab 77:1393-1397.

Ghigo E 1992 Neurotransmitter control of growth hormone secretion. In: De la Cruz LF (ed) Regulation of growth hormone and somatic growth. Excerpta Medica, Amsterdam, p.103.

Ghigo E, Goffi S, Mazza E, Arvat E, Procopio M, Bellone J, Muller E E, Camanni F 1989 Repeated GH-releasing hormone administration unravels different GH secretory patterns in normal adults and children. Acta Endocrinologica 120:598-601.

Ghigo E, Arvat E, Gianotti L, Imbimbo B P, Lenaerts V, Deghenghi R, Camanni F 1994a Growth hormone-releasing acitivity of hexarelin, a new synthetic hexapeptide, after intravenous, subcutaneous, intranasal, and oral administration in man. J Clin Endocrinol Metab 78:693-698.

Ghigo E, Arvat E, Rizzi G, Nicolosi M, Boffano G M, Mucci M, Boghen M F, Camanni F 1994b Arginine enhances the growth hormone-releasing activity of a synthetic hexapeptide (GHRP-6) in elderly but not in young subjects after oral administration. J Endocrinol Invest 17:157-162.

Ghigo E, Arvat E, Rizzi G, Goffi S, Grottoli S, Mucci M, Boghen M F, Camanni F 1994c Growth hormone-releasing activity of growth hormone-releasing peptide-6 is maintained after short-term oral pretreatment with the hexapeptide in normal aging. Eur J Endocrinol 131:499-503.

Ghigo E, Arvat E, Gianotti L, Grottoli S, Rizzi G, Ceda G P, Boghen M F, Deghenghi R, Camanni F 1996a Short-term administration of intranasal or oral Hexarelin, a synthetic hexapeptide, does not desensitize the growth hormone responsiveness in human aging. Eur J Endocrinol 135:407-412.

Ghigo E, Bellone J, Aimaretti G, Bellone S, Loche S, Cappa M, Bartolotta E, Dammacco F, Camanni F 1996b Reliability of provocative tests to assess growth hormone secretory status. Study in 472 normally growing children. J Clin Endocrinol Metab 81:3323-3327.

Giustina A, Girelli A, Doga M, Bodini C, Bossoni S, Romanelli G, Wehrenberg W B 1990 Pyridostigmine blocks the inhibitory effect of glucocorticoids on growth hormone-releasing hormone-stimulated growth hormone secretion in normal men. J Clin Endocrinol Metab 71:580-584.

Giustina A, Girelli A, Alberti D, Bossoni S, Buzi F, Doga M, Schettino M, Wehrenberg W B 1991 Effects of pyridostigmine on spontaneous and growth hormone-releasing hormone stimulated growth hormone secretion in children on daily glucocorticoid therapy after liver transplantation. Clin Endocrinol 35:491-498.

Giustina A, Wehrenberg W B 1992 Role of glucocorticoids in the regulation of growth hormone secretion: mechanisms and clinical significance. Trends Endocrinol Metab 3:306-311.

Giustina A, Bussi A R, Deghenghi R, Imbimbo B P, Licini M, Poiesi C, Wehrenberg W B 1995 Comparison of the effects of growth hormone-releasing hormone and hexarelin, a novel growth hormone-releasing peptide-6 analog, on growth hormone secretion in humans with or without glucocorticoid excess. J Endocrinol 146:227-232.

Glass A R, Burman K D, Dahms W T, Boehm T M 1981 Endocrine function in human obesity. Metabolism 30:89-104.

Goth M I, Lyons C E, Canny B J, Thorner M O 1992 Pituitary adenylate cyclase activating polypeptide, growth hormone (GH)-releasing peptide and GH-releasing hormone stimulate GH release through distinct pituitary receptors. Endocrinology 130:939-944.

Guillaume V, Magnan E, Cataldi M, Dutour A, Sauze N, Renard M, Razafindraibe H, Conte-Devolx B, Deghenghi R, Lenaerts V, Oliver C 1994 Growth hormone (GH)-releasing hormone secretion is stimulated by a new GH-releasing hexapeptide in sheep. Endocrinology 135:1073-1076.

Hanew K, Utsumi A, Sugawara A, Shimizu Y, Abe K 1994 Enhanced GH responses to combined adminstration of GHRP and GHRH in patients with acromegaly. J Clin Endocrinol Metab 78:509-512.

Hansen T B, Vahl N, Jorgensen J O L, Christiansen J S, Hagen C 1995 Whole body and regional soft tissue changes in growth hormone deficient adults after one year of growth hormone treatment: a double-blind, randomized, placebo-controlled study. Clin Endocrinol 43:689-696.

Hartman M L, Farello G, Pezzoli S S, Thorner M O 1992 Oral administration of growth hormone (GH)-releasing peptide stimulates GH secretion in normal men. J Clin Endocrinol Metab 74:1378-1384.

Hayashi S, Okimura Y, Yagi H, Uchiyama T, Takeshima Y, Shakutsui S, Oohashi S, Bowers C Y, Chihara K 1991 Intranasal administration of His-D-Trp-Ala-Trp-D-Phe-LysNH_2 (growth hormone releasing peptide) increased plasma growth hormone and IGF-1 levels in normal men. Endocrinol Japon 38:15-21.

Herrington J, Hille B 1994 Growth hormone-releasing hexapeptide elevates intracellular calcium in rat somatotropes by two mechanisms. Endocrinology 135:1100-1108.

Hindmarsh P C, Matthews D R, Brain C E, Pringle P J, Di Silvio L, Kurtz A B, Brook C G D 1989 The half-life of exogenous growth hormone after suppression of endogenous growth hormone secretion with somatostatin. Clin

Endocrinol 30:443-450.

Hindmarsh P C, Matthews D R, Brain C E, Pringle P J, Brook C G D 1990 The application of deconvolution analysis to elucidate the pulsatile nature of growth hormone secretion using a variable half-life of growth hormone. Clin Endocrinol 32:739-747.

Hindmarsh P C, Brain C E, Robinson I C A F, Matthews D R, Brook C G D 1991 The interaction of growth hormone releasing hormone and somatostatin in the generation of a GH pulse in man. Clin Endocrinol 35:353-360.

Ho P J, Kletter G B, Hopwood N J, DeMott-Friberg R, Barkan A L 1993 Somatostatin withdrawal alone is an ineffective generator of pulsatile growth hormone release in man. Acta Endocrinologica 129:414-418.

Howard A D, Feighner S D, Cully D F, Arena J P, Liberator P A, Rosenblum C I, Hamelin M, Hreniuk D L, Palyha O C, Anderson J, Paress P S, Diaz C, Chou M, Liu K K, McKee K K, Pong S-S, Chaung L-Y, Elbrecht A, Dashkevicz M, Heavens R, Rigby M, Sirinathsinghji D J S, Dean D C, Melillo D G, Patchett A A, Nargund R, Griffin P R, DeMartino J A, Gupta S K, Schaeffer J M, Smith R G, Van der Ploeg L H T 1996 A receptor in pituitary and hypothalamus that functions in growth hormone release. Science 273:974-977.

Hughes J, Smith T W, Kosterlitz H W, Fothergill L A, Morgan B A, Morris H R 1975 Identification of two related pentapeptides from the brain with potent opiate agonist activity. Nature 258:577-579.

Huhn W C, Hartman M L, Pezzoli S S, Thorner M O 1993 Twenty-four-hour growth hormone (GH)-releasing peptide (GHRH) infusion enhances pulsatile GH secretion and specifically attenuates the response to a subsequent GHRP bolus. J Clin Endocrinol Metab 76:1202-1208.

Ilson B E, Jorkasky D K, Curnow R T, Stote R M 1989 Effect of a new synthetic hexapeptide to selectively stimulate growth hormone release in healthy human subjects. J Clin Endocrinol Metab 69:212-214.

Imaki T, Shibasaki T, Shizume K, Masuda A, Hotta M, Kiyosawa Y, Jibiki K, Demura H, Tsushima T, Ling N 1985 The effect of free fatty acids on growth hormone-releasing hormone-mediated GH secretion in man. J Clin Endocrinol Metab 60:290-293.

Imbimbo B P, Mant T, Edwards M, Amin D, Dalton N, Boutignon F, Lenaerts V, Wüthrich P, Deghenghi R 1994 Growth hormone-releasing activity of hexarelin in humans: a dose-response study. Eur J Clin Pharmacol 46:421-425.

Jacks T, Hickey G, Judith F, Taylor J, Chen M H, Krupa D A, Feeney W, Schoen W R, Ok D, Fisher M H 1994 Effects of acute and repeated intravenous administration of L-692,585, a novel non-peptidyl growth hormone secretagogue, on plasma growth hormone, IGF-1, ACTH, cortisol, prolactin,

insulin, and thyroxine levels in beagles. J Endocrinol 143:399-406.

Jaffe C A, Ho P J, Demott-Friberg R, Bowers C Y, Barkan A L 1993 Effects of a prolonged growth hormone (GH)-releasing peptide infusion on pulsatile GH secretion in normal men. J Clin Endocrinol Metab 77:1641-1647.

Jaffe C A, DeMott-Friberg R, Barkan A L 1996 Endogenous growth hormone (GH)-releasing hormone is required for GH responses to pharmacological stimuli. J Clin Invest 97:934-940.

Jansson J-O, Downs T R, Beamer W G, Frohman L A 1986a The dwarf 'little' (*lit/lit*) mouse is resistant to growth hormone (GH)-releasing peptide (GH-RP-6) as well as the GH-releasing hormone (GRH). Proceedings of the 68th Annual Meeting of The American Endocrine Society, 1986. 397 (abstract).

Jansson J-O, Downs T R, Beamer W G, Frohman L A 1986b Receptor associated resistance to growth hormone-releasing hormone in dwarf 'little' mice. Science 232:511-512.

Knopf R F, Conn W J, Fajans S S, Floyd J C, Guntsche E M, Rull J A 1965 Plasma growth hormone response to intravenous administration of amino acids. J Clin Endocrinol Metab 25:1140-1144.

Koch B D, Blalock J B, Schonbrunn A 1988 Characterisation of the cyclic AMP-independent actions of somatostatin in GH cells. An increase in potassium conductance is responsible for both the hyperpolarisation and the decrease in intracellular free calcium produced by somatostatin. J Biol Chem 263:216-225.

Korbonits M, Trainer P J, Besser G M 1995 The effect of an opiate antagonist on the hormonal changes induced by hexarelin. Clin Endocrinol 43:365-371.

Kraicer J, Cowan J S, Sheppard M S, Lussier B T, Moor B C 1986 Effects of somatostatin withdrawal and growth hormone (GH)-releasing factor on GH release *in vitro*: amount available for release after disinhibition. J Endocrinol 119:2047-2051.

Laron Z, Bowers C Y, Hirsch D, Almonte A S, Pelz M, Keret R, Gil-Ad I 1993 Growth hormone-releasing activity of growth hormone-releasing peptide-1 (a synthetic heptapeptide) in children and adolescents. Acta Endocrinologica 129:424-426.

Laron Z, Frenkel J, Gil-Ad I, Klinger B, Lubin E, Wüthrich P, Boutignon F, Lenaerts V, Deghenghi R 1994 Growth hormone releasing activity by intranasal administration of a synthetic hexapeptide (Hexarelin). Clin Endocrinol 41:539-541.

Laron Z, Frenkel J, Deghenghi R, Anin S, Klinger B, Silbergeld A 1995a Intranasal administration of the GHRP hexarelin accelerates growth in short children. Clin

Endocrinol 43:631-635.

Laron Z, Frenkel J, Eshet R, Klinger B, Silbergeld A 1995b Human growth hormone and insulin-like growth factor-1 stimulating and growth promoting effects by long term intranasal administration of the hexapeptide-hexarelin. Proceedings of the 77th Annual Meeting of The American Endocrine Society, Washington DC, 1995. 504 (abstract P3-141).

Leal-Cerro A, Pumar A, Garcia-Garcia E, Dieguez C, Casanueva F F 1994 Inhibition of growth hormone release after the combined administration of GHRH and GHRP-6 in patients with Cushing's syndrome. Clin Endocrinol 41:649-654.

Leal-Cerro A, Garcia E, Astorga R, Casanueva F F, Dieguez C 1995 Growth hormone (GH) responses to the combined administration of GH-releasing hormone plus GH-releasing peptide-6 in adults with GH deficiency. Eur J Endocrinol 132:712-715.

Lei T, Buchfelder M, Fahlbusch R, Adams E F 1995 Growth hormone releasing peptide (GHRP-6) stimulates phosphatidylinositol (PI) turnover in human pituitary somatotroph cells. J Mol Endocrinol 14:135-138.

Leonhardt S, Jarry H, Kreipe A, Werstler K, Wuttke W 1992 Pituitary adenylate cyclase-activating polypeptide (PACAP) stimulates pituitary hormone release in male rats. Neuroendocrinol Lett 14:319-328.

Li C H, Chung D, Doneen B A 1976 Isolation, characterisation and opiate activity of β-endorphin from human pituitary glands. Biochem Biophys Res Commun 72:1542-1547.

Lin S C, Lin C R, Gukovsky I, Lusis A J, Sawchenko P E, Rosenfeld M G 1993 Molecular basis of the little mouse phenotype and implications for cell-specific growth. Nature 364:208-213.

Ling N, Esch F, Bohlen P, Brazeau P, Wehrenberg W B, Guillemin R 1984 Isolation, primary structure and synthesis of human hypothalamic somatocrinin; growth hormone-releasing factor. Proc Natl Acad Sci USA 81:4302-4306.

Locatelli V, Grilli R, Torsello A, Cella S G, Wehrenberg W B, Muller E E 1994 Growth hormone-releasing hexapeptide is a potent stimulator of growth hormone gene expression and release in the growth hormone-releasing hormone-deprived infant rat. Pediatr Res 36:169-174.

Loche S, Cambiaso P, Carta D, Setzu S, Imbimbo B P, Borrelli P, Pintor C, Cappa M 1995a The growth hormone-releasing activity of hexarelin, a new synthetic hexapeptide, in short normal and obese children and in hypopituitary subjects. J Clin Endocrinol Metab 80:674-678.

Loche S, Cambiaso P, Merola B, Colao A, Faedda A, Imbimbo B P, Deghenghi R,

Lombardi G, Cappa M 1995b The effect of hexarelin on growth hormone (GH) secretion in patients with GH deficiency. J Clin Endocrinol Metab 80:2692-2696.

Locke W, Kirgis H D, Bowers C Y, Abdoh A A 1995 Intracerebroventricular growth-hormone-releasing peptide-6 stimulates eating without affecting plasma growth hormone responses in rats. Life Sci 56:1347-1352.

Maccario M, Arvat E, Procopio M, Gianotti L, Grottoli S, Imbimbo B P, Lenaerts V, Deghenghi R, Camanni F, Ghigo E 1995 Metabolic modulation of the growth hormone-releasing activity of hexarelin in man. Metabolism 44:134-138.

Mallo F, Alvarez C V, Benitez L, Burguera B, Coya R, Casanueva F F, Dieguez C 1993 Regulation of His-dTrp-Ala-Trp-dPhe-Lys-NH_2-induced GH secretion in the rat. Neuroendocrinology 57:247-256.

Malozowski S, Hao E H, Ren S G, Marin G, Liu L, Southers J L, Merriam G R 1991 Growth hormone (GH) responses to the hexapeptide GH-releasing peptide and GH-releasing hormone (GHRH) in the cynomolgus macaque: evidence for non-GHRH-mediated responses. J Clin Endocrinol Metab 73:314-317.

Martha P M, Rogol A D, Veldhuis J D, Kerrigan J R, Goodman D W, Blizzard R M 1989 Alterations in the pulsatile properties of circulating growth hormone concentration during puberty in boys. J Clin Endocrinol Metab 69:563-570.

Mason W T, Dickson S L, Leng G 1993 Control of growth hormone secretion at the single cell level. Acta Paediatr 388(suppl.):84-92.

Massara F, Ghigo E, Molinatti P, Mazza E, Locatelli V, Camanni F 1986 Potentiation of cholinergic tone by pyridostigmine bromide re-instates and potentiates the growth hormone responsiveness to intermittent administration of growth hormone releasing factor in man. Acta Endocrinologica 13:12-16.

Mau S E, Witt M R, Bjerrum O J, Saermark T, Vilhardt H 1995 Growth hormone releasing hexapeptide (GHRP-6) activates the inositol (1,4,5)-triphosphate/diacylglycerol pathway in rat anterior pituitary cells. J Rec Sig Trans Res 15:311-323.

Mauras N, Blizzard R M, Link K, Johnson M L, Rogol A D, Veldhuis J D 1987 Augmentation of growth hormone secretion during puberty: evidence for a pulse amplitude-modulated phenomenon. J Clin Endocrinol Metab 64:596-601.

McCormick G F, Millard W J, Badger T M, Bowers C Y, Martin J B 1985 Dose-response characteristics of various peptides with growth hormone-releasing activity in the unanesthetized male rat. Endocrinology 117:97-105.

McDowell R S, Elias K A, Stanley M S, Burdick D J, Butnier J P, Chan K S, Fairbrother W J, Hammonds R G, Ingle G S, Jacobsen N E 1995 Gowth hormone secretagogues: characterization, efficacy, and minimal bioactive

conformation. Proc Natl Acad Sci USA 92:11165-11169.

Melmed S 1991 Medical progress: acromegaly. N Engl J Med 322:966-977.

Mericq V, Cassorla F, Garcia H, Avila A, Bowers C Y, Merriam G R 1995a Growth hormone (GH) responses to GH-releasing peptide and to GH-releasing hormone in GH-deficient children. J Clin Endocrinol Metab 80:1681-1684.

Mericq F, Cassorla F, Salazar T, Avila A, Iniguez G, Bowers C Y, Merriam G R 1995b Increased growth velocity during prolonged GHRP-2 administartion to growth hormone deficient children. Proceedings of the 77th annual meeting of The American Endocrine Society, 1995. 85 (abstract OR30-3).

Micic D, Popovic V, Kendereski A, Macut D, Casanueva F F, Dieguez C 1995 Growth hormone secretion after the administration of GHRP-6 or GHRH combined with GHRP-6 does not decline in late adulthood. Clin Endocrinol 42:191-194.

Micic D, Popovic V, Kendereski A, Peino R, Dieguez C, Casanueva F F 1996 The sequential administration of growth hormone-releasing hormone followed 120 minutes later by hexarelin, as an effective test to assess the pituitary GH reserve in man. Clin Endocrinol 45:543-551.

Miki N, Ono M, Shizume K 1988 Withdrawal of endogenous somatostatin induces secretion of growth hormone-releasing factor in rats. J Endocrinol 117:245-252.

Momany F A, Bowers C Y, Reynolds G A, Chang D, Hong A, Newlander K 1981 Design, synthesis, and biological activity of peptides which release growth hormone *in vitro*. Endocrinology 108:31-39.

Momany F A, Bowers C Y, Reynolds G A, Hong A, Newlander K 1984 Conformational energy studies and *in vitro* and *in vivo* activity data on growth hormone-releasing peptides. Endocrinology 114:1531-1536.

Muccioli G, Ghe C, Ghigo M C, Arvat E, Papotti M, Boghen M F, Deghenghi R, Ong H, Ghigo E 1995 Presence of specific receptors for hexarelin, a GH-releasing hexapeptide, in human brain and pituitary gland. Proceedings of the 20th International Symposium on Growth and Growth Factors in Endocrinology and Metabolism, Berlin, 1995. 110 (abstract).

Muruais J, Peñalva A, Dieguez C, Casanueva F F 1993 Influence of endogenous cholinergic tone alpha-adrenergic pathways on growth hormone responses to His-D-Trp-Ala-Trp-D-Phe-Lys-NH_2 in the dog. J Endocrinol 138:211-218.

Narayanan N, Lussier B T, French M, Moor B C, Kraicer J 1989 Growth hormone-releasing factor-sensitive adenylate cyclase system of purified somatotrophs: effects of guanine nucleotides, somatostatin, calcium and magnesium. Endocrinology 124:484-492.

Nastainczyk W, Rohrkasten A, Sieber M, Rudolph C, Schachtele C, Marme D, Hofmann F 1987 Phosphorylation of the purified receptor for calcium channel blockers by cAMP kinase and protein kinase C. Eur J Biochem 169:137-142.

Nelson A H, Walker R F, Codd E E, Barone F C 1991 Intranasal activity of the growth hormone releasing peptide His-D-Trp-Ala-Trp-D-Phe-Lys-NH_2 in conscious dogs. Life Sci 48:2283-2288.

Nishizuka Y 1988 The molecular heterogeneity of protein kinase C and its implication for cellular regulation. Nature 334:661-665.

Nyborg J K, Nguyen A P, Spindler S R 1984 Relationship between thyroid and glucocorticoid hormone receptor occupancy, growth hormone gene transcription and mRNA accumulation. J Biol Chem 259:12377-12381.

Patchett A A, Nargund R P, Tata J R, Chen M H, Barakat K J, Johnston D B, Cheng K, Chan W W-S, Butler B, Hickey G 1995 Design and biological activities of L-163,191 (MK-0677): a potent, orally active growth hormone secretagogue. Proc Natl Acad Sci USA 92:7001-7005.

Peñalva A, Pombo M, Carballo A, Barreiro J, Casanueva F F, Dieguez C 1993a Influence of sex, age and adrenergic pathways on the growth hormone response to GHRP-6. Clin Endocrinol 38:87-91.

Peñalva A, Carballo A, Pombo M, Casanueva F F, Dieguez C 1993b Effects of growth hormone (GH)-releasing hormone (GHRH), atropine, pyridostigmine, or hypoglycaemia on GHRP-6-induced GH secretion in man. J Clin Endocrinol Metab 76:168-171.

Pihoker C, Middleton R, Reynolds G A, Bowers C Y, Badger T M 1995a Diagnostic studies with intravenous and intranasal growth hormone-releasing peptide-2 in children of short stature. J Clin Endocrinol Metab 80:2987-2992.

Pihoker C, Bowers C Y, Middleton R, Reynolds G A, Badger T M 1995b Treatment effects of intranasal growth hormone releasing peptide-2 (GHRP-2) in growth hormone deficient children. Proceedings of the 77th Annual Meeting of The American Endocrine Society, Washington, DC, 1995. 504 (abstract P3-143).

Pombo M, Barreiro J, Peñalva A, Mallo F, Casanueva F F, Dieguez C 1995a Plasma growth hormone response to growth hormone-releasing hexapeptide (GHRP-6) in children with short stature. Acta Paediatr 84:904-908.

Pombo M, Barreiro J, Peñalva A, Peino R, Dieguez C, Casanueva F F 1995b Absence of growth hormone (GH) secretion after the administration of either GH-releasing hormone (GHRH), GH-releasing peptide (GHRP-6), or GHRH plus GHRP-6 in children with neonatal pituitary stalk transection. J Clin Endocrinol Metab 80:3180-3184.

Pong S-S, Chaung L-Y P, Smith R G 1991 GHRP-6

(His-D-Trp-Ala-Trp-D-Phe-Lys-NH_2) stimulates growth hormone secretion by depolarization in rat pituitary cell cultures. Proceedings of the 73rd Annual Meeting of The American Endocrine Society, Washington, DC, 1991. 88 (abstract).

Pong S-S, Chaung L-Y P, Leonard R J 1993 The involvement of ions in the activity of a novel growth hormone secretagogue L-692,429 in rat pituitary cell cultures. Proceedings of the 75th Annual Meeting of The American Endocrine Society, Las Vegas, 1993. 172 (abstract).

Pong S-S, Chaung L-Y P, Dean D C, Nargund R P, Patchett A A, Smith R G 1996 Identification of a new G-protein-linked receptor for growth hormone secretagogues. Mol Endocrinol 10:57-61.

Popovic V, Damjanovic S, Micic D, Petakov M, Dieguez C, Casanueva F F 1994 Growth hormone (GH) secretion in active acromagaly after combined administration of GH-releasing hormone and GH-releasing peptide-6. J Clin Endocrinol Metab 79:456-460.

Popovic V, Damjanovic S, Micic D, Djurovic M, Dieguez C, Casanueva F F 1995 Blocked growth hormone-releasing peptide (GHRP-6)-induced GH secretion and absence of the synergic action of GHRP-6 plus GH-releasing hormone in patients with hypothalamopituitary disconnection: evidence that GHRP-6 main action is exerted at the hypothalamic level. J Clin Endocrinol Metab 80:942-947.

Prader A, Zachmann M, Poley J R, Illig R, Szeky J 1967 Long term treatment with human growth hormone (Raben) in small doses. Evaluation of 18 hypopituitary children. Acta Paediatr Helv 22:423-439.

Ramos-Dias J C, Pimentel-Filho F, Reis A F, Lengyel A-M J 1996 Different growth hormone (GH) responses to GH-releasing peptide and GH-releasing hormone in hyperthyroidism. J Clin Endocrinol Metab 81:1343-1346.

Rawlings S R, Hezareh M 1996 Pituitary Adenylate Cyclase-Activating Polypeptide (PACAP) and PACAP/Vasoactive Intestinal Polypeptide receptors: actions on the anterior pituitary gland. Endocr Rev 17:4-29.

Renner U, Brockmeier S, Strasburger C J, Lange M, Schopohl J, Muller O A, Werder K V, Stalla G K 1994 Growth hormone (GH)-releasing peptide stimulation of GH release from human somatotroph adenoma cells: interaction with GH-releasing hormone, thyrotropin-releasing hormone, and octreotide. J Clin Endocrinol Metab 78:1090-1096.

Rivier J, Spiess J, Thorner M O, Vale W 1982 Characterisation of a growth hormone releasing factor from a human pancreatic islet tumour. Nature 300:276-278.

Robinson B M, Friberg R D, Bowers C Y, Barkan A L 1992 Acute growth hormone (GH) response to GH-releasing hexapeptide in humans is independent

of endogenous GH-releasing hormone. J Clin Endocrinol Metab 75:1121-1124.

Rosenfeld R G, Albertsson-Wikland K, Cassorla F, Frasier S D, Hasegawa Y, Hintz R L, Lafranchi S, Lippe B, Loriaux L, Melmed S, Preece M A, Ranke M B, Reiter E O, Rogol A D, Underwood L E, Werther G A 1995 Diagnostic controversy: the diagnosis of childhood growth hormone deficiency revisited. J Clin Endocrinol Metab 80:1532-1540.

Roth J, Glick S M, Yalow R S, Berson S A 1963 Hypoglycemia: a potent stimulus of growth hormone. Science 140:987-988.

Roumi M, Lenaerts V, Boutignon F, Wüthrich P, Deghenghi R, Bellemare M, Adam A, Ong H 1995 Radioimmunoassay for hexarelin, a peptidic hormone secretagogue, and its pharmacokinetic studies. Peptides 16:1301-1306.

Rudman D, Feller A G, Nagraj H S, Gergans G A, Lalitha P Y, Goldberg A F, Schlenker R A, Cohn L, Rudman I W, Mattson D E 1990 Effects of human growth hormone in men over 60 years old. N Engl J Med 323:1-6.

Saini S, Hindmarsh P C, Matthews D R, Pringle P J, Jones J, Preece M A, Brook C G D 1991 Reproducibility of 24-hour serum growth hormone profiles in man. Clin Endocrinol 34:455-462.

Saito H, Inoue T, Fukatsu K, Ming-Tsan L, Fukushima R, Muto T 1996 Growth hormone and the immune response to bacterial infection. Horm Res 45:50-54.

Sartor A O, Bowers C Y, Chang D 1985 Parallel studies of His-D-Trp-Ala-Trp-DPhe-Lys-NH_2 and human pancreatic growth hormone-releasing factor-44-NH_2 in rat primary pituitary cell monolayer culture. Endocrinology 116:952-957.

Sartorio A, Conti A, Ferrero S, Spada A, Faglia G 1995 GH responsiveness to repeated GHRH or hexarelin administration in normal adults. J Endocrinol Invest 18:718-722.

Sethumadhavan K, Veeraragavan K, Bowers C Y 1991 Demonstration and characterization of the specific binding of growth hormone-releasing peptide to rat anterior pituitary and hypothalamic membranes. Biochem Biophys Res Commun 178:31-37.

Shibasaki T, Shizume K, Nakahara M, Masuda A, Jibiki K, Demura H, Wakabayashi I, Ling N 1984 Age-related changes in plasma growth hormone response to growth hormone-releasing factor in man. J Clin Endocrinol Metab 58:212-214.

Sims S M, Lussier B T, Kraicer J 1991 Somatostatin activates an inwardly rectifying K^+ conductance in freshly dispersed rat somatotrophs. J Physiol (Lond) 441:615-637.

Smith R G, Cheng K, Schoen W R, Pong S-S, Hickey G, Jacks T, Butler B, Chan W W-S, Chaung L-Y P, Judith F, Taylor J, Wyvratt M J, Fisher M H 1993 A nonpeptidyl growth hormone secretagogue. Science 260:1640-1643.

Smith S L, Hindmarsh P C, Brook C G D 1993 Compliance with growth hormone treatment-are they getting it? Arch Dis Child 68:91-93.

Soto J L, Castrillo J-L, Dominguez F, Dieguez C 1995 Regulation of the pituitary-specific transcription factor GHF-1/Pit-1 messenger ribonucleic acid levels by growth hormone-secretagogues in rat anterior pituitary cells in monolayer culture. Endocrinology 136:3863-3870.

Spoudeas H A, Matthews D R, Brook C G D, Hindmarsh P C 1992 The effect of changing somatostatin tone on the pituitary growth hormone and thyroid-stimulating hormone responses to their respective releasing factor stimuli. J Clin Endocrinol Metab 75:453-458.

Spoudeas H A, Winrow A P, Hindmarsh P C, Brook C G D 1994 Low-dose growth hormone-releasing tests: a dose-response study. Eur J Endocrinol 131:238-245.

Stewart J M, Young S D 1968 Solid Phase Peptide Synthesis. Freeman, San Francisco.

Stojilkovic S S, Catt J K 1992 Calcium oscillation in anterior pituitary cells. Endocr Rev 13:256-280.

Suri D, Hindmarsh P C, Matthews D R, Brain C E, Brook C G D 1991 The pituitary gland is capable of responding to two successive doses of growth hormone releasing hormone (GHRH). Clin Endocrinol 34:13-17.

Tannenbaum G S, Painson J-C, Lapointe M, Gurd W, McCarthy G F 1990 Interplay of somatostatin and growth hormone-releasing hormone in genesis of episodic growth hormone secretion. Metabolism 9:35-39.

Tiulpakov A N, Brook C G D, Pringle P J, Peterkova V A, Volevodz N N, Bowers C Y 1995a GH responses to intravenous bolus infusions of GH releasing hormone and GH releasing peptide-2 separately and in combination in adult volunteers. Clin Endocrinol 43:347-350.

Tiulpakov A N, Bulatov A A, Peterkova V A, Elizarova G P, Volevodz N N, Bowers C Y 1995b Growth hormone (GH)-releasing effects of synthetic peptide GH-releasing peptide-2 and GH-releasing hormone (1-29)NH_2 in children with GH insufficiency and idiopathic short stature. Metabolism 44:1199-1204.

Torsello A, Panzeri A, Cermenati P, Caroleo M C, Ghigo E, Camanni F, Muller E E, Locatelli V 1988 Involvement of the somatostatin and cholinergic systems in the mechanism of growth hormone autofeedback regulation in the rat. J

Endocrinol 117:273-281.

Tuomisto J, Mannisto P 1985 Neurotransmitter regulation of anterior pituitary hormones. Pharmacol Rev 37:249-332.

Turner R C, Grayburn J A, Newman G B, Nabarro J D N 1971 Measurement of the insulin delivery rate in man. J Clin Endocrinol Metab 33:279-286.

Valcavi R, Zini M, Portiolo I 1992 Thyroid hormones and growth hormone secretion. J Endocrinol Invest 15:313-330.

Valcavi R, Dieguez C, Zini M, Muruais C, Casanueva F F, Portiolo I 1993 Influence of hyperthyroidism on growth hormone secretion. J Clin Endocrinol Metab 38:515-522.

Van den Berghe G, de Zegher F, Vlasselaers D, Schetz M, Verwaest C, Ferdinande P, Lauwers P 1996a Thyrotropin releasing hormone in critical illness: from a dopamine-dependent test to a strategy for increasing low serum triiodothyronine, prolactin and growth hormone. Crit Care Med 24:542-549.

Van den Berghe G, de Zegher F, Bowers C Y, Wouters P, Muller P, Soetens F, Vlasselaers D, Schetz M, Verwaest C, Lauwers P, Bouillon R 1996b Pituitary responsiveness to GH-releasing hormone, GH-releasing peptide-2 and thyrotrophin-releasing hormone in critical illness. Clin Endocrinol 45:341-351.

Veeraragavan K, Sethumadhavan K, Bowers C Y 1992 Growth hormone-releasing peptide (GHRP) binding to porcine anterior pituitary and hypothalamic membranes. Life Sci 50:1149-1155.

Voerman H J, Strack van Schijndel R J, Groeneveld A B, de Boer H, Nauta J P, Van der Veen E A, Thijs L G 1992 Effects of recombinant human growth hormone in patients with severe sepsis. Ann Surg 216:648-655.

Voerman B J, Strack van Schijndel R J, Groeneveld A B, de Boer H, Nauta J P, Thijs L G 1995 Effects of human growth hormone in critically ill nonseptic patients: results from a prospective, randomised, placebo-controlled trial. Crit Care Med 23:665-673.

Walker R F, Codd E E, Barone F C, Nelson A H, Goodwin T, Campbell S A 1990 Oral activity of growth hormone relesing peptide His-D-Trp-Ala-Trp-D-Phe-Lys-NH_2 in rats, dogs, and monkeys. Life Sci 47:29-36.

Wehrenberg W B, Ling N 1983 *In vivo* biological potency of rat and human growth hormone releasing factor and fragments of human growth hormone releasing factor. Biochem Biophys Res Commun 115:525-530.

Williams T, Berelowitz M, Joffe S N, Thorner M O, Rivier J, Vale W, Frohman L A 1984 Impaired growth hormone responses to growth hormone releasing factor in

obesity. N Engl J Med 311:1403-1407.

Willoughby J, Brogan M, Kapoor R 1989 Hypothalamic interconnections of somatostatin and growth hormone releasing factor on growth hormone secretion. Neuroendocrinology 50:584-591.

Wu D, Chen C, Katoh K, Zhang J, Clarke I J 1994 The effects of GH-releasing peptide-2 (GHRP-2 or KP 102) on GH secretion from primary cultured ovine pituitary cells can be abolished by a specific GH-releasing factor (GRF) receptor antagonist. J Endocrinol 140:R9-R13.

Wu D, Chen C, Bowers C Y, Clarke I J 1996 The effects of GH-releasing peptide-6 (GHRP-6) and GHRP-2 on intracellular adenosine 3',5'-monophosphate (cAMP) levels and GH secretion in ovine and rat somatotrophs. J Endocrinol 148:197-205.

Yaffe B M, Samuels H 1984 Hormonal regulation of the growth hormone gene: relationship of the rate of transcription to the level of nuclear thyroid hormone-receptor complexes. J Biol Chem 259:6284-6291.

Yagi H, Uchiyama T, Shakutsui S, Chihara K 1990 Modulation by endogenous GHRH and SRIF of GH releasing hexapeptide (GHRP-6) induced GH release in conscious male rats. Proceedings of the 72nd Annual Meeting of The American Endocrine Society, 1990. 50 (abstract).

Yamashita S, Melmed S 1986 Insulin-like growth factor-I action on rat anterior pituitary cells: suppression of growth hormone secretion and messenger ribonucleic acid levels. Endocrinology 118:176-182.

Ziegler T R, Young L S, Ferrari-Baliviera E, Demling R H, Wilmore D W 1990 Use of human growth hormone combined with nutritional support in a critical care unit. J Parenter Enteral Nutr 14:574-581.

PUBLICATIONS ARISING FROM THIS THESIS

1. Massoud AF, Hindmarsh PC, Brook CGD (1995). Hexarelin-induced growth hormone release is influenced by exogenous growth hormone. *Clinical Endocrinology* 43; 617-621.

2. Massoud AF, Hindmarsh PC, Matthews DR, Brook CGD (1996). The effect of repeated administration of hexarelin, a growth hormone releasing peptide, and growth hormone releasing hormone (GHRH) on growth hormone (GH) responsivity. *Clinical Endocrinology* 44; 555-562.

3. Massoud AF, Hindmarsh PC, Brook CGD (1996). Hexarelin-induced growth hormone, cortisol and prolactin release: a dose-response study. *Journal of Clinical Endocrinology and Metabolism* 81; 4338-4341.

4. Massoud AF, Hindmarsh PC, Brook CGD (1997). Interaction of the growth hormone releasing peptide hexarelin with somatostatin. *Clinical Endocrinology* (In Press).