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Human Growth Hormone*

JEANNINE S. STROBL† AND MICHAEL J. THOMAS

Department of Pharmacology and Toxicology, Robert C. Byrd Health Sciences Center, West Virginia University, Morgantown, West Virginia, and Departments of Medicine and Biochemistry and Molecular Biophysics, Washington University School of Medicine, Washington University Medical Center, St. Louis, Missouri

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† Address for correspondence: Department of Pharmacology and Toxicology, Robert C. Byrd Health Sciences Center, P.O. Box 9223, West Virginia University, Morgantown, WV 26506.

I. Introduction

A. Historical Perspective

The first clinical observations regarding the function of the pituitary gland are credited to Pierre Marie in 1886, who noted enlargement of the pituitary gland in an acromegalic patient. Investigations by Dr. Harvey Cushing at the Johns Hopkins Hospital during the early 1900s yielded the first experimental data attributing the function of the anterior pituitary to growth in humans, mice, and dogs (Cushing, 1909a,b; 1910; Crowe et al., 1910).

In 1921, Evans and Long showed that saline extracts of bovine anterior pituitary promoted growth in normal rats (Evans and Long, 1921), and soon after, it was found that such extracts could restore growth in hypophysectomized rats (Smith, 1927). The growth-promoting extracts of bovine pituitary were also found to exert general anabolic effects, resulting in decreased urinary excretion of nitrogen, calcium, and phosphorus (Cuthbertson et al., 1941a,b). The measurement of these elements in urine provided a convenient bioassay and facilitated the isolation of the active component in pituitary extracts. Li and Evans (1944) first isolated the peptide hormone now known as growth hormone from bovine pituitaries.

Because bovine growth hormone failed to promote human growth (Bennett et al., 1950), the isolation of human growth hormone was undertaken using human pituitary glands obtained either at autopsy or from patients with breast cancer treated by hypophysectomy (Li and Papkoff, 1956; Li, 1957; Raben, 1957). In clinical trials, both human and monkey growth hormone were anabolic and stimulated longitudinal growth (Li, 1957; Raben, 1958). Biochemical differences between primate and nonprimate growth hormones have been identified and account for the species specificity of the physiological effects (Li, 1957).

Between 1957 and 1985, human growth hormone isolated from human cadaveric pituitaries was used as replacement therapy in many growth hormone-deficient children (reviewed by Frasier, 1983). During this time, growth hormone therapy was found to be effective and remarkably free of undesirable side effects. However, human pituitary growth hormone was scarce and expensive. The exploration for other therapeutic applications or the cellular mechanisms of human growth hormone action was not possible.

B. New Biochemical Developments

Several advances have changed the human growth hormone field recently. The DNA encoding human growth hormone was cloned in bacterial plasmids (Martial et al., 1979), opening the way to the large-scale commercial production of human growth hormone using recombinant DNA technology. The commercial development of recombinant human growth hormone, as well as its ready acceptance into clinical usage, was spurred by a 1985 report of an increased incidence of Creutzfeldt-Jakob disease, a fatal degenerative neurological disorder, among adults who had received human growth hormone during childhood (Brown et al., 1985). It has been demonstrated that some human growth hormone preparations were contaminated with the slow virus that caused Creutzfeldt-Jakob disease (Gibbs et al., 1993). Although it is now generally accepted that viral contamination is preventable, a recent outbreak of Creutzfeldt-Jakob disease in France among growth hormone-deficient individuals who received human pituitary growth hormone reinforces the value of recombinant human growth hormone for replacement therapy (Aldhous, 1992). With the large-scale production of human growth hormone by bacteria, the supply of human growth hormone no longer restricts the types or numbers of experimental therapies in humans or laboratory studies that can be conducted.

More recently, the human growth hormone receptor protein was purified, and its gene was molecularly cloned (Leung et al., 1987; Wallis, 1987). The three-dimensional structure of the human growth hormone receptor-growth hormone interaction was elucidated by x-ray crystallography (de Vos et al., 1992). Knowledge of the structure of the growth hormone receptor has provided new insight and direction to research concerning growth hormone signal transduction mechanisms. Site-directed mutagenesis has been performed in an effort to elucidate precise structural requirements for the growth hormone ligand-receptor interaction (Cunningham and Wells, 1989, 1991; Fuh et al., 1992). Molecular approaches such as these are being applied to the design of growth hormone receptor agonists with more selective physiological actions as well as to the design of receptor antagonists. Other areas of active research include the elucidation of the physiological role of human growth hormone structural variants (reviewed by Baumann, 1987) and the function of human growth hormone serum-binding proteins, which appear to be secreted forms of the human growth hormone receptor (reviewed by Carlsson et al., 1991; Herrington et al., 1991; Postel-Vinay and Fontoura, 1991).

C. New Therapeutic Approaches

It is now widely recognized that most human growth deficiencies are due to hypothalamic defects that impair the release of pituitary growth hormone and are not the result of a primary deficit in the production of growth hormone by the pituitary. As a result, the development of synthetic growth hormone-releasing agents and the use of drugs acting through established neurotransmitter systems in the brain to stimulate growth hormone release are being considered as alternatives to highly expensive growth hormone replacement therapy for the restoration of normal serum growth hormone levels.

II. Growth Hormone Synthesis

A. Human Growth Hormone Gene Family

The human growth hormone gene cluster spans approximately 66,500 bases of DNA on the long arm of chromosome 17 (reviewed by Hirt et al., 1987; Miller and Eberhardt, 1983; Parks, 1986). The five closely related genes that make up this gene family are *GH-N* (growth hormone-normal gene), *CS-L* (chorionic somatomammotropin-like), *CS-A* (chorionic somatomammotropin-A), *GH-V* (growth hormone-variant gene; also placental growth hormone), and *CS-B* (chorionic somatomammotropin-B) (fig. 1). The *GH-N* and *GH-V* genes are also sometimes referred to as *GH-1* and *GH-2*, respectively. The chorionic somatomammotropin genes are also referred to as placental lactogen genes (*PL-L*, *PL-A*, and *PL-B*, respectively). The DNA sequence of these five genes as well as their associated intervening sequences and 5′-flanking sequences is highly homologous. This has led to the hypothesis that the growth hormone gene family arose by gene duplication events.

Only the *GH-N* gene is required for normal health. Deletion or mutation of the *GH-N* gene results in isolated growth hormone deficiency type IA and is the most severe form of growth hormone deficiency. Individuals with this deficiency express no endogenous growth hormone. Severe hypoglycemia and growth delays are evident at birth

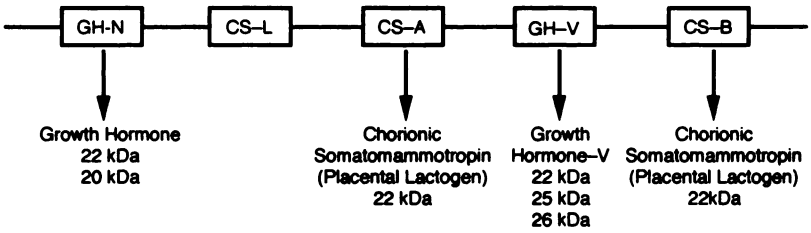

FIG. 1. Human growth hormone gene cluster.

or within 3 to 6 months of birth (Akinci et al., 1992; Cogan et al., 1993; Phillips et al., 1981, 1982; reviewed by Phillips et al., 1986). These patients initially respond to growth hormone replacement but develop resistance to treatment within 1 year because of growth hormone antibody production.

Other inherited forms of isolated growth hormone deficiency include isolated growth hormone deficiency types IB, II, and III. Individuals with these diseases express low levels of growth hormone. Mutations in the growth hormone locus have not been identified in these inherited forms of isolated growth hormone deficiency (reviewed by Phillips et al., 1986).

B. Growth Hormone and Related Peptides

1. GH-N gene products. The *GH-N* gene is expressed primarily in the anterior pituitary gland and is responsible for the major circulating form of human growth hormone in children and adults (reviewed by Baumann, 1991). Growth hormone is a secreted protein and is synthesized as a prehormone containing a 26-amino acid signal sequence. Cleavage (processing) of the signal sequence occurs as the hormone is transferred across the rough endoplasmic reticulum into storage granules. The processed protein product of the *GH-N* gene is a single chain, 22-kDa, 191-amino acid peptide. The biologically active conformation of human growth hormone is maintained by two disulfide bridges. The 22-kDa *GH-N* gene product binds receptors in human liver with high affinity and stimulates IGF-I‡ production and linear growth.

The RNA product from the *GH-N* gene also undergoes alternative splicing, resulting in a second growth hormone translation product of 20 kDa. The 20-kDa human growth hormone variant lacks 15 internal amino acids, residues 32 to 46, compared with the 22-kDa form. Approximately 5 to 15% of circulating immunoreactive human growth hormone is the 20-kDa human growth hormone variant.

The 22- and 20-kDa forms of human growth hormone may have different biological activities. Both the 22- and 20-kDa products of the *GH-N* gene are capable of stimulating growth in transgenic mice (Stewart et al., 1992). However, the 20-kDa form does not bind with high affinity to growth hormone receptors in human liver, and its ability to stimulate growth in humans is unknown. Some investigators have reported that the 20-kDa growth hormone form has less insulin-like activity than the 22-kDa form of human growth hormone (Culler et al., 1988; Frigeri et al., 1979). The *GH-N* gene is also expressed in human lymphoid cells, where it may modulate immune function. Lymphocytes express receptors with higher affinity for the 20- than the 22-kDa form of human growth hormone (Smal et al., 1985).

2. GH-V gene products. Four genes in the growth hormone gene cluster, including *GH-V,* are expressed in the placenta (Hill, 1992; MacLeod et al., 1992). The *GH-V* gene product is found at very high concentrations in the maternal circulation during pregnancy. In fact, the *GH-V* gene product replaces that of *GH-N* as the principal form of circulating growth hormone during the latter half of pregnancy. The physiological significance of this replacement has not been elucidated. Normal pregnancy and growth can occur in the complete absence of the *GH-V* gene product because of gene deletion (Akinci et al., 1992; Wurzel et al., 1982).

The *GH-V* gene product cannot substitute for the *GH-N* gene product in stimulating linear growth in children (Phillips et al., 1982). The *GH-N* and *GH-V* gene products differ by 13 amino acids that are dispersed throughout the polypeptide chain. These amino acid substitutions have a marked effect on the isoelectric point of the protein: 5.5 and 8.9 for *GH-N* and *GH-V,* respectively (Parks, 1986). Three protein products of the *GH-V* gene have been identified in the circulation: (*a*) the expected 22-kDa gene product, hGH-V; (*b*) a 25-kDa glycosylated form of *GH-V*; and (*c*) a 26-kDa form denoted hGH-V2 (Hill, 1992).

3. CS-A and CS-B gene products. The *CS-A* and *CS-B* genes are expressed in human placenta and encode an identical protein, human chorionic somatomammotropin or placental lactogen (Barrera-Saldena et al., 1983). Production of the *CS-A, CS-B,* and *GH-V* gene products is blocked in the pituitary by repressor proteins that bind to a 5′-flanking sequence common to these three genes (Nachtigal et al., 1993).

Placental lactogen is thought to be involved in the growth of the fetus, mammary gland development, and lactogenesis; however, completely normal offspring have been born in the absence of placental lactogen (Nielsen

‡ Abbreviations: IGF, insulin-like growth factor; GRF, growth hormone-releasing factor; SRIF, somatotropin release-inhibiting factor; L-DOPA, levodopa; LDL, low-density lipoprotein; GHRP, growth hormone-releasing peptide.

et al., 1979; Wurzel et al., 1982). It is possible that other members of the growth hormone gene family can function in the place of placental lactogen. The amino acid sequence of placental lactogen is 87% identical with the 22-kDa protein product of the *GH-N* gene.

The *CS-L* gene is a pseudogene and does not produce a functional protein.

4. Posttranslational modifications of human growth hormone. Growth hormone heterogeneity has been reviewed (Baumann, 1991; Lewis, 1992). When the human pituitary content of growth hormone is analyzed immunologically, a complex mixture of growth hormone peptides is revealed. This complexity is derived from posttranslationally modified forms of human growth hormone and differs markedly from the homogeneous preparations of 22-kDa human growth hormone produced biosynthetically in bacteria from the human growth hormone cDNA. Approximately 75% of the growth hormone in the human pituitary occurs in an unmodified form; only 43% of growth hormone in the circulation exists as the unmodified 22-kDa form of human growth hormone. All animal species examined have also exhibited a similar degree of growth hormone heterogeneity. The physiological function of the modified growth hormone forms is not known.

Approximately 5% of the physiological forms of modified growth hormone that appear in the circulation are acidic forms of the monomeric 22-kDa human growth hormone: desamido-human growth factor (22-kDa Asp-152 and 22-kDa Glu-137) and acylated human growth factor-22 kDa. About 20% of circulating growth hormone occurs as a dimer of the 22-kDa gene product, and about 7% is present in higher oligomeric forms of the 22-kDa gene product (trimers-pentamers). The majority of the remaining modified forms of growth hormone in the circulation consists of monomeric, dimeric, or oligomeric forms of the 20-kDa gene product. Now that these immunologically reactive forms of human growth hormone have been biochemically characterized, it may be possible to assess their individual roles in growth hormone physiology.

C. Regulation of Growth Hormone Synthesis

1. Developmental expression. Growth hormone synthesis is detectable in the human fetal pituitary as early as 12 weeks' gestation. The level of growth hormone in the fetal circulation at 12 weeks is about 20 ng/ml and increases to a maximum of approximately 80 ng/ml at 22 weeks' gestation. Growth hormone levels in the fetus decline at term to approximately 10 ng/ml (Grumbach and Kaplan, 1973). Growth hormone receptors are present in the fetal liver and may mediate growth hormone stimulation of hepatocyte proliferation and IGF-I synthesis. Normal or near-normal linear growth can be achieved in the fetus in the absence of growth hormone, however. The high levels of circulating growth hormone may reflect active production of GRF preceding the maturation of somatostatin-producing cells (Hill, 1992).

2. Growth hormone messenger RNA regulation in somatotrophs. Levels of growth hormone mRNA in human somatotrophs are under the control of general and tissue-specific transcription factors, hormones, and cAMP (fig. 2).

a. PITUITARY-SPECIFIC TRANSCRIPTION FACTOR PIT-1 (GROWTH HORMONE FACTOR-1). Genes that direct organ differentiation during embryonic development are called homeotic genes. The protein products of homeotic genes are often referred to as homeodomain proteins (reviewed by Rosenfeld, 1991). Pit-1 (also known as growth hormone factor-1) is a homeodomain protein that is essential for pituitary differentiation and growth hormone expression in humans and rodents. Pit-1 protein stimulates proliferation of pituitary somatotrophs and is essential for transcription of the *GH-N* gene in rodents (Castrillo et al., 1991). The onset of growth hormone mRNA transcription in the pituitary during embryogenesis in rodents occurs simultaneously with the expression of the pituitary-specific transcription factor Pit-1 (Simmons et al., 1990).

The importance of Pit-1 in humans has been documented clinically. Several patients with growth retardation and low growth hormone synthesis have been found to carry inactivating mutations in the *Pit-1* gene (reviewed by Parks et al., 1993; Pfaffle et al., 1992; Radovick et al., 1992; Tatsumi et al., 1992). The Pit-1 protein binds to two specific DNA regions immediately upstream of the transcription initiation site in the human *GH-N* gene. The binding of Pit-1 to these sites activates mRNA transcription of the *GH-N* gene, and mutations within the Pit-1 DNA-binding sites that prevent protein binding inhibit gene transcription (Lefevre et al., 1987).

Pit-1 protein also plays an important role in the stimulation of growth hormone production by cAMP. cAMP regulates gene expression through the binding to CREB, a cAMP response element-binding protein, which binds to specific DNA sequences and stimulates gene transcription. The promoter region for the rat *Pit-1* gene contains DNA-binding sites for the cAMP-CREB complex. Increasing intracellular cAMP levels in transformed rat pituitary cells in tissue culture stimulates Pit-1 transcription. It is the cAMP-stimulated increase in Pit-1 protein that contributes to the cAMP stimulation of growth hormone mRNA expression. Deletion or mutation of the Pit-1-binding sites upstream of the human growth hormone gene blunts the response to cAMP (McCormick et al., 1990).

Pit-1 and cAMP are involved in the stimulation of growth hormone mRNA synthesis by GRF in rat pituitary cells (Barinaga et al., 1985; Dana and Karin, 1989). GRF stimulates an acute increase in the intracellular levels of cAMP in somatotroph cells. A region within the first 82 nucleotides upstream of the human growth hor-

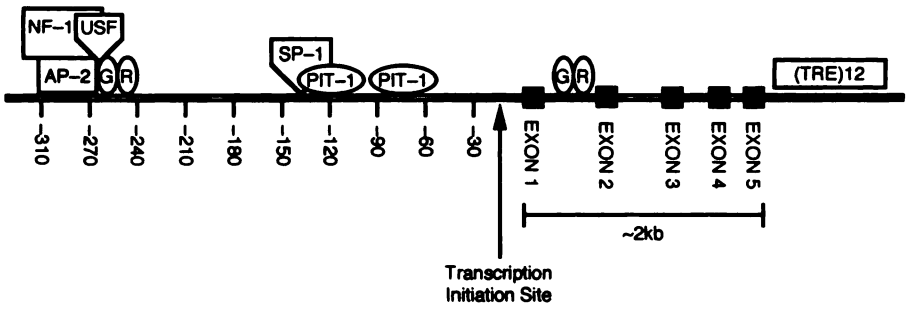

FIG. 2. Transcriptional regulation of the human *GH-N* gene. AP-2, activator protein-2, NF-1, USF/MLTF, upstream stimulating factor/major late transcription factor.

mone gene transcription initiation site is required for the induction of mRNA expression by cAMP and GRF. The response to GRF is reduced when the two DNA-binding sites for Pit-1 (between 82 and 120 nucleotides upstream of the transcription initiation site) are eliminated (Dana and Karin, 1989).

b. GENERAL TRANSCRIPTION FACTORS. Proteins that participate in mRNA transcription of many different genes in multiple cell types are general transcription factors (reviewed by Latchman, 1990; Mitchell and Tijan, 1989; Pabo and Sauer, 1992). Protein-protein interactions among transcription factors are common and interactions between different general transcription factors as well as interactions between general transcription factors, tissue-specific factors, and hormone-receptor complexes all play a role in modulating levels of mRNA. DNA-binding sites for four general transcription factors have been identified in the human growth hormone gene upstream region: Sp1, activator protein-2, nuclear factor-1, and upstream stimulating factor/major late transcription factor. Sp1 and Pit-1 recognize overlapping DNA sequences in the human growth hormone gene upstream region, as do activator protein-2 and nuclear factor-1. Human growth hormone gene expression may be regulated by competition for DNA binding by these different transcription factors (Courtois et al., 1990; Lemaigre et al., 1990).

c. GLUCOCORTICOIDS. Glucocorticoids increase levels of human growth hormone mRNA by increasing gene transcription (Slater et al., 1985) and increasing mRNA stability (Paek and Axel, 1987). Glucocorticoid stimulation of gene transcription is mediated by the binding of glucocorticoid hormone-receptor complexes to specific DNA sequences called glucocorticoid response elements. Two glucocorticoid response elements have been identified in the human growth hormone gene: one occurs in the region upstream of the transcription initiation site, and the second one is located in the first intron of the gene (Moore et al., 1985; Slater et al., 1985). The glucocorticoid response element within the first intron of the gene binds glucocorticoid-receptor complexes most avidly and induces a 3-fold stimulation of growth hormone gene transcription. The glucocorticoid response element upstream of the gene may have some slight stimulatory effect on gene transcription.

The mechanism whereby glucocorticoids increase growth hormone mRNA stability is not known. Sequences within the coding region of the gene are required for this response (Paek and Axel, 1987).

d. THYROID HORMONE. Thyroid hormone decreases levels of human growth hormone mRNA. The DNA sequences responsible for the effect of thyroid hormone lie within the first 88 nucleotides immediately downstream of the human growth hormone translation stop codon. This region contains 12 copies of the hexanucleotide half-site (A/GGGNNN) that make up the thyroid hormone response element (Zhang et al., 1992). Thyroid hormone does not influence the mRNA stability. It has been hypothesized that these downstream elements decrease mRNA expression by inhibiting transcription initiation or by blocking mRNA elongation.

D. Signal Transduction Pathways Involved in Growth Hormone Secretion

Growth hormone is stored in secretory granules within the somatotroph cells of the anterior pituitary until a stimulus for secretion occurs. Secretion is stimulated by increasing intracellular concentrations of cAMP and/or free Ca^{2+}. GRF, the most important physiological growth hormone secretagogue, acts via GRF receptors coupled to G-α subunit s (Gaylinn et al., 1993; Mayo et al., 1992; Spada et al., 1992). GRF binding to somatotroph cells results in increased cAMP production via the G-protein-mediated stimulation of adenylate cyclase (Bilezikjian and Vale, 1983; Sundberg et al., 1976). Somatostatin opposes the action of GRF on cAMP levels in somatotroph cells (Bilezikjian and Vale, 1983; Sundberg et al., 1976). Somatostatin receptors are coupled to G-α subunit i, and receptor occupancy results in the inhibition of adenylate cyclase (Spada et al., 1992).

Increases in the intracellular concentration of free Ca^{2+} are associated with increased growth hormone secretion. In somatotroph cells, the concentration of free Ca^{2+} is regulated by calcium entry through voltage-activated L-type calcium channels. Membrane depolarization allows calcium influx through these L-type calcium

channels. GRF and elevations in intracellular cAMP stimulate Ca^{2+} influx through voltage-sensitive calcium channels (Lussier et al., 1991a). The effects of both GRF and forskolin on growth hormone secretion are blocked by dihydropyridine calcium channel blockers.

Somatostatin, the most important inhibitor of growth hormone secretion, stimulates potassium ion efflux, causing membrane hyperpolarization and inhibition of growth hormone secretion. At least two types of potassium channels have been implicated in the control of membrane potential in rat pituicytes: the large-conductance potassium channel activated by calcium ion and voltage (White et al., 1991) and the ATP-sensitive potassium channel (De Weille et al., 1992). Membrane hyperpolarization antagonizes calcium entry via the voltage-sensitive calcium channels. Thus, a reduction in the concentration of free intracellular Ca^{2+} contributes to the inhibition of growth hormone secretion by somatostatin (Lussier et al., 1991b).

III. Growth Hormone Physiology

A. Human Growth Hormone Receptor

The cDNA for the human growth hormone receptor was isolated by screening a human cDNA library with the rabbit growth hormone receptor cDNA (Leung et al., 1987). The human growth hormone receptor cDNA encodes a 638-amino acid polypeptide which includes an 18 amino acid signal peptide and a mature full-length receptor of 620 amino acids. Hydropathy plots of the amino acid sequence reveal a single transmembrane domain with an extracellular growth hormone-binding domain of 246 amino acids and a cytoplasmic domain 350 amino acids long. The predicted molecular weight based on amino acid sequence is approximately 70 kDa, but the observed molecular weight based on gel electrophoresis is 109 kDa in human IM-9 lymphocytes (Hughes et al., 1983) and 124 kDa in human liver (Hocquette et al., 1990). The apparent discrepancy may be explained by posttranslational modifications, such as N-glycosylation and covalently bound ubiquitin, a 76-amino acid protein. At least five potential N-glycosylation sites exist on the extracellular domain. Although glycosylation does not appear to be necessary for ligand binding (de Vos et al., 1992; Fuh et al., 1990), it may direct transport and insertion of the receptor into the cell membrane (Urbanek et al., 1992).

The human growth hormone receptor shares a high degree of homology with the amino acid sequences of other species (>70%), including rabbit, bovine, porcine, ovine, rat, and mouse growth hormone receptors. The human growth hormone receptor displays 30% amino acid sequence homology (50% based on conserved substitutions) with the prolactin receptor. The growth hormone and prolactin receptors are part of a much larger cytokine receptor family that includes erythropoietin, colony-stimulating factors (granulocyte and granulocyte-macrophage), interleukins 2, 3, 4, 5, 6, and 7, and a glycoprotein that associates with interleukin-6, gp130. Other more distantly related members of this family include the interferon receptors (α, β, γ), and ciliary neurotrophic factor (reviewed by Kelly et al., 1991). All of these receptors exhibit varying degrees of homology among their extracellular domains, which is termed the cytokine receptor homologous domain, but these receptors vary significantly among their cytoplasmic domains. Key features of this receptor family include two disulfide bond linkages at the NH_2-terminal region of the extracytoplasmic domain which may participate in the formation of ligand-binding pockets for each particular receptor. These receptors also have a WSXWS motif (tryptophan-serine-any amino acid-tryptophan-serine), except for the growth hormone receptor (in which there are conservative substitutions in the WSXWS motif: YGZFS) (de Vos et al., 1992). The function of the WSXWS motif is not known but may permit the interaction of the receptor with the ligand or other components of the receptor complex.

X-ray crystallography of the extracellular domain of the human growth hormone receptor has revealed that the receptor forms a dimer when binding a single growth hormone molecule (de Vos et al., 1992) (fig. 3). This finding correlates with stoichiometry observed in solution (Cunningham et al., 1991) and reveals that there are two binding sites on each growth hormone molecule that are recognized by essentially the same determinants on the growth hormone receptor. Electrostatic interactions between the separate extracellular domain molecules are weak, suggesting that binding of the ligand is necessary for dimerization, and this dimerization may be necessary to induce growth hormone receptor signal transduction (de Vos et al., 1992).

Growth hormone receptor mRNA is present in many tissues within the rat, as summarized in table 1. Ontologically, rat growth hormone receptor mRNA levels are low in all tissues at birth, but increase in the postnatal period, most prominently in liver, kidney, heart, and muscle (Matthews et al., 1989). Regulation of growth hormone receptor gene expression is complex but may be modulated by growth hormone (Bick et al., 1992; Nilsson et al., 1990), although this has not been observed in the rat in vivo (Matthews et al., 1989). Other physio-

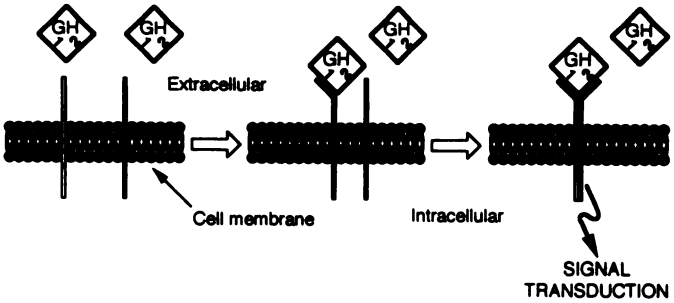

FIG. 3. Dimerization of growth hormone receptor.

TABLE 1
Tissue distribution of growth hormone receptor mRNA in the rat

Liver
Heart
Kidney
Intestine
Lung
Muscle
Pancreas
Brain
Testes

logical states, such as pregnancy, can increase growth hormone receptor mRNA levels (Matthews et al., 1989).

The gene for the human growth hormone receptor appears to be on chromosome 5, adjacent to the gene for the prolactin receptor. The human growth hormone gene spans at least 87 kilobases and is composed of at least 10 exons (Godowski et al., 1989). Multiple transcripts of varying length from various species have been isolated, but in human liver, a 4.5-kilobase mRNA transcript predominates, although other smaller transcripts have been observed. Alternative splicing of the gene transcripts occurs, which may give rise to mRNAs that encode for growth hormone-binding proteins in mice and rats (Baumbach et al., 1989); however, in humans and rabbits, growth hormone-binding proteins appear to arise through proteolytic cleavage (Carlsson et al., 1991).

Growth hormone is transported through the circulation by at least two binding proteins (Baumann, 1991; Herrington et al., 1991). A high-affinity, low-capacity binding protein (GH-BP-1) has a molecular weight of 61 kDa and bears structural/immunological resemblance to the extracellular domain of the growth hormone receptor. Interestingly, African Pygmies have low levels of this high-affinity growth hormone-binding protein, which may partially explain their short stature (Baumann et al., 1989). A low-affinity, high-capacity binding protein (GH-BP-2) has a molecular weight of about 100 kDa and is not structurally related to GH-BP-1 or the growth hormone receptor. GH-BP-2 exhibits a higher specificity for the 20-kDa growth hormone variant (Baumann, 1991, and references therein). The mechanism by which growth hormone-binding proteins modulate growth hormone action is unclear but may influence the proportion of free (active) versus bound (inactive) growth hormone, or the binding proteins may play a role in growth hormone clearance from the circulation.

Laron dwarfism is a rare inherited form of short stature that has recently been shown to occur as a result of mutations in the growth hormone receptor gene (Laron, 1993; Goossens et al., 1993). Most reported cases of Laron dwarfism occur in the Middle East. Molecular genetic analysis has identified exon deletion mutations (Godowski et al., 1989) and base substitutions (Amselem et al., 1989). Cases of Laron dwarfism in Ecuador appear to be the result of a substitution at codon 180 of exon 6, resulting in a splice shift in the growth hormone receptor gene (Rosenbloom, 1992; Rosenbloom et al., 1990). In Laron dwarfs, growth hormone levels are normal, but both IGF-I and growth hormone-binding protein levels are low. Treatment of affected individuals with growth hormone is ineffective, although IGF-I treatment can modestly augment growth velocity (Wilton et al., 1992).

B. Growth Hormone Receptor Signal Transduction

Mutational analysis of truncated growth hormone receptors has revealed that as few as 54 amino acids of the 350 amino acid cytoplasmic domain are capable of transmitting a growth hormone proliferative signal in a promyeloid cell line (Colosi et al., 1993). The growth hormone receptor is thought to be associated with a tyrosine kinase that is activated when growth hormone binds to the receptor (Wang et al., 1992). This tyrosine kinase has been identified as JAK-2, a member of a family of recently identified tyrosine kinases. Following growth hormone binding and receptor dimerization, an intracellular protein of approximately 120 kDa that is physically associated with the activated receptor undergoes tyrosine phosphorylation (Stred et al., 1990). In addition, the receptor itself (Wang et al., 1993) and other cellular proteins become tyrosine phosphorylated, including the 42- and 45-kDa mitogen-activated protein kinases (or extracellular signal-regulated protein kinases) (Anderson, 1992; Campbell et al., 1992; Winston and Bertics, 1992). Mitogen-activated protein kinases comprise a family of intracellular signaling molecules that have been implicated in a broad array of biological responses and are modified by a variety of extracellular signals (Pelech and Sanghera, 1992).

Growth hormone also triggers the rapid induction of early response genes c-*fos* and c-*jun* (Minami et al., 1992; Slootweg et al., 1991). These effects occur through activation of protein kinase C and in 3T3F442A adipocytes appear to proceed through a signaling pathway that is distinct from that involved in phosphorylation of mitogen-activated protein kinases (Gurland et al., 1990). Other investigators have suggested a role for protein kinase C activation in growth hormone signaling. Both diacylglycerol and inositol trisphosphate (via the release of intracellular calcium) activate protein kinase C. Growth hormone activates phospholipase C in canine renal tubules, resulting in inositol trisphosphate and diacylglyerol production (Rogers and Hammerman, 1989). In tissue culture cells, a similar effect on diacylglycerol production (but not inositol trisphosphate) has been noted (Slootweg et al., 1991).

C. Neuroendocrine Regulation of Growth Hormone Secretion via the Hypophyseal-Pituitary Axis

Green and Harris (1947) described a portal pituitary blood supply that was densely innervated by cell bodies located within the hypothalamus. They proposed that this unique neuroanatomical arrangement was the basis for the control of pituitary hormone secretion by the

hypothalamus. We now know that growth hormone secretion from the anterior pituitary gland is principally regulated by two hypothalamic peptides that are released into this portal circulation. GRF, also known as growth hormone-releasing hormone or somatocrinin, is the hormone responsible for hypothalamic stimulation of growth hormone secretion. Somatostatin or SRIF is the peptide primarily responsible for inhibition of growth hormone secretion from the pituitary gland.

1. Growth hormone-releasing factor. Human GRF was first isolated and characterized from GRF-hypersecreting pancreatic tumors (Guillemin et al., 1982; Rivier et al., 1982) and was subsequently identified in the hypothalamus (Bohlen et al., 1983). Human GRF is synthesized as a 107- or 108-amino acid precursor protein and undergoes proteolytic processing and posttranslational modification to two forms. GRF(1–40) is a 40-amino acid form, and GRF(1–44NH2) is a 44-amino acid form containing an amidated NH_2 terminus. The synthesis of GRF in humans takes place in cell bodies primarily located in the arcuate nucleus of the basal hypothalamus. Cells extend to the periventricular zone and partially overlap both the tuberoinfundibular and ventromedial nuclei (Bloch et al., 1984). Following secretion into the portal blood, GRF reacts with receptors on the membrane of pituitary somatotrophs to stimulate growth hormone secretion and transcription of new growth hormone mRNA (Barinaga et al., 1983).

2. Somatostatin. Somatostatin or SRIF was first identified in extracts of ovine hypothalamus (Brazeau et al., 1973). In 1980, Schally et al. and Esch et al. determined the 28-amino acid sequence of somatostatin-28. The structure of human somatostatin was deduced from the sequence of its cDNA and is identical with that found in sheep and pig hypothalami (Shen et al., 1982).

There are a number of structurally related somatostatin peptides with biological activity. Somatostatin-28 and somatostatin-14 contain 28 and 14 amino acids, respectively; they are identical at their carboxy termini (Shen et al., 1982). Both somatostatin-28 and somatostatin-14 inhibit growth hormone secretion. These forms of somatostatin exhibit some differences in tissue distribution, however, and are localized preferentially within the gastrointestinal tract (somatostatin-28) and brain (somatostatin-14). Secreted high molecular weight forms of somatostatin also exist (Devesa et al., 1992; Reichlin, 1992).

Distinct cell bodies in the hypothalamus produce GRF and SRIF. SRIF synthesis occurs primarily in the periventricular region in humans, although additional SRIF-producing cell bodies have been identified by immunocytochemistry in the preoptic area, suprachiasmic nucleus, and lateral hypothalamus (Gabriel et al., 1987). SRIF suppresses growth hormone release in two ways. First, neurons in the preoptic area release SRIF at GRF-producing hypothalamic cells, thus suppressing GRF release. Second, SRIF is secreted into the portal circulation and suppresses growth hormone release from the pituitary following binding to receptors on somatotrophs in the anterior pituitary.

3. Pulsatile secretion of growth hormone. Pulsatile secretion of growth hormone is a hallmark of normal hypothalamic-pituitary function (Dierschke et al., 1970; reviewed by Brook and Hindmarsh, 1992; Frohman and Jansson, 1986; Iranmanesh and Veldhuis, 1992; Martin, 1978; Muller, 1987). GRF and SRIF are essential in all animal species, including humans, for pulsatile growth hormone secretion. In rodents, pulsatile growth hormone secretion is driven by the oscillatory release of GRF and SRIF, 180 degrees out of phase. Growth hormone release from the anterior pituitary occurs primarily in response to GRF stimulation when SRIF levels are low. The pituitary response to GRF is blunted if SRIF levels are elevated (Plotsky and Vale, 1985; Tannenbaum and Ling, 1984; Wehrenberg et al., 1982a). In humans, most evidence suggests that somatostatin is principally responsible for the pulsatile timing of growth hormone secretion (reviewed by Thorner et al., 1990). Pulsatile secretion of growth hormone in humans is correlated with oscillatory reductions in serum SRIF levels, even in the face of continuously elevated GRF levels (Vance et al., 1985). The magnitude of the growth hormone response to GRF is also modulated by changes in the sensitivity of the pituitary somatotroph cells to somatostatin. Somatostatin and growth hormone down-regulate pituitary somatostatin receptor levels, attenuating the inhibitory effects of somatostatin at the level of the pituitary. This, in turn, enhances the responsiveness of the pituitary to GRF.

Pulsatile release of growth hormone occurs in neonates, children, adolescents, and adults and is believed to play an important, but as yet undefined, role in the physiological actions of growth hormone (Goji, 1993; Miller et al., 1993). Pulse frequencies ranging from slightly more than one growth hormone pulse per hour (Holl et al., 1991) to one pulse every 4 hours have been reported (Casanueva, 1992). The higher frequency of release has been obtained in studies in which more frequent sampling has been performed, suggesting that pulses of growth hormone release are missed when sampling frequency is less intensive.

In rodents, pulsatile exposure to growth hormone results in more pronounced physiological effects than does continuous administration of the same total dosage (Isgaard et al., 1988). Similar observations have been made in children in whom the longitudinal growth response to human growth hormone replacement therapy was greater when the identical dose was administered in divided doses three to four times weekly rather than once per week (Frasier, 1983).

In general, physiological conditions resulting in higher 24-hour growth hormone serum levels are associated with

increased growth hormone pulse amplitudes rather than with increased pulse frequency. These include sleep, exercise, and puberty. Similarly, reductions in serum growth hormone seen with aging and in individuals with short stature are associated with a decreased pulse amplitude with no change in pulse frequency. The mechanisms controlling growth hormone pulse amplitude vary among physiological states as detailed in the following sections.

a. SLEEP-INDUCED SECRETION OF GROWTH HORMONE. Secretion of growth hormone occurs primarily during sleep. During a 24-hour period, peak levels of serum growth hormone occur during the first episode of slow-wave sleep, followed by additional peaks of growth hormone secretion that are coincident with subsequent waves of slow-wave sleep (Holl et al., 1991; Takahashi et al., 1968). In humans, evidence that sleep-induced growth hormone secretion is biologically significant has been obtained by correlating height in children with the magnitude of the growth hormone peak that occurs during the first slow-wave sleep episode (Bercu, 1987).

It has been proposed that cortical centers that regulate the onset of slow-wave sleep simultaneously direct the release of growth hormone from the pituitary (Holl et al., 1991). In the rat, stimulation of muscarinic cholinergic nerves within the hypothalamus increases growth hormone secretion via the inhibition of SRIF release (Richardson et al., 1980; Locatelli et al., 1986). This cholinergic pathway is likely to be involved in the sleep-induced increase in growth hormone secretion because muscarinic antagonists prevent sleep-associated increases in plasma growth hormone levels (Mendelson et al., 1978).

More recently, it has been suggested that GRF and/or growth hormone are humoral mediators of sleep (Kerkhofs et al., 1993; Krueger and Obal, 1993). This hypothesis explains the association of growth hormone secretion and sleep in young adults and also offers an explanation for the poor sleeping patterns typically seen in elderly individuals. GRF and growth hormone administration can induce slow-wave sleep in humans, but this effect is dependent on the time of day growth hormone is given. Under certain circumstances, growth hormone administration induces REM sleep.

b. EXERCISE-INDUCED SECRETION OF GROWTH HORMONE. Exercise is one of the most effective stimuli of growth hormone secretion. Exercise-induced growth hormone secretion is enhanced by estrogens. The magnitude of response to exercise is greater in women than in men and is maximal in women during periods of peak serum estrogen levels (Frantz and Rabkin, 1965; Merimee et al., 1969). An acute increase in plasma growth hormone begins within 10 minutes of onset of strenuous exercise and reaches a maximum after 40 to 60 minutes of continuous exertion (Bunt et al., 1986; Raynaud et al., 1983; Sutton and Lazarus, 1976; VanHelder et al., 1987). The amount of growth hormone secreted increases with exercise intensity. Maximum exercise-stimulated plasma growth hormone levels range between 35 and 60 ng/ml and return to resting daytime levels of 1 to 2 ng/ml within 30 minutes following cessation of exercise.

In various studies, the effects of exercise training on the acute release of growth hormone in response to exercise have been examined with mixed results. In some reports, trained individuals exhibited a blunted growth hormone response to exercise (Galbo, 1983) and, in others, training enhanced the release of growth hormone relative to controls (Bunt et al., 1986). Because the results of such studies are dependent on the level of training in the trained group and the condition of the control group as well as level of exercise intensity, it is not surprising that conflicting results were obtained. Perhaps of greater interest is the effect of training on resting patterns of growth hormone secretion. Weltman et al. (1992) conducted a study of 24-hour growth hormone secretion profiles in a group of women before and after 1 year of endurance training. In this study, strenuous training resulted in nearly a doubling of growth hormone secretion that was manifest as an increase in basal (interpeak) plasma growth hormone levels and an increased growth hormone pulse height. There was no change in growth hormone pulse frequency.

c. GROWTH HORMONE CHANGES DURING PUBERTY. Rapid longitudinal growth among 10- to 13-year-old girls and 12- to 15-year-old boys in the North American population is characteristic of puberty (reviewed by Brook and Hindmarsh, 1992; Martha and Reiter, 1991; Kerrigan and Rogol, 1992). Growth hormone is essential for growth during this time. Elevations in the 24-hour serum growth hormone levels during puberty are the result of marked increases in the growth hormone pulse amplitude. Neither increases in the pulse frequency nor decreases in the rate of growth hormone clearance from serum have been noted. In addition to growth, growth hormone plays a role in gonadal maturation during puberty (Stanhope et al., 1992) and the development of secondary sexual characteristics in boys (Zachmann, 1992).

Growth stimulation by growth hormone during puberty is interrelated with elevated gonadal steroid hormone secretion, particularly that of estradiol. Estrogens, and to a lesser extent androgens, stimulate GH secretion from the pituitary. Estrogens also modify end-organ responsiveness to growth hormone. The effects of estrogens are dose related. Low doses of estrogens exert a stimulatory effect on growth via the stimulation of growth hormone release from the pituitary, whereas high doses of estrogens inhibit growth via inhibition of growth hormone effects in the bone (Zachmann et al., 1975).

The role of estradiol in growth has been demonstrated most clearly in estrogen-deficient females with Turner's syndrome. Without supplemental estrogen, patients with

Turner's syndrome rarely achieve an adult height greater than 4 feet, 9 inches (Lyon et al., 1985). Yet, no height differences are evident between normal prepupertal girls and prepupertal patients with Turner's syndrome. Estrogen administration to girls of pubertal age with Turner's syndrome stimulates secretion of growth hormone in an amplified pulsatile profile characteristic of that seen in normal girls undergoing puberty (Mauras et al., 1989). Estrogens do not simply stimulate growth hormone secretion, however, because hormone replacement in patients with Turner's syndrome stimulates growth synergistically when estrogens and growth hormone are used in cotreatment.

Growth hormone levels and linear growth rates are elevated in girls exhibiting precocious puberty. Control of ovarian estradiol secretion with gonadotropin-releasing hormone antagonists has been reported to return growth and growth hormone secretion patterns to normal (Harris et al., 1985; Mansfield et al., 1988). These results point to an important regulatory role for estradiol in growth hormone secretion. However, in another study, leuprolide acetate (a gonadotropin-releasing hormone antagonist) reduced linear growth rates and circulating estradiol levels with no measurable reductions in serum growth hormone or IGF-I levels (Sklar et al., 1991). The results of this later study indicate that direct effects of estradiol on bone growth contribute to growth in precocious puberty. Collectively, the results of clinical studies of precocious puberty are in agreement with those of patients with Turner's syndrome, i.e., there are synergistic effects of estrogens and growth hormone on adult stature.

The mechanism of estrogen action on growth hormone secretion in humans is not well-defined, although a role for endogenous opioids has been implicated by several investigators (Wilson et al., 1991). Intravenous injection of enkephalin analogs stimulates an acute increase in plasma growth hormone (Stubbs et al., 1978), and ovarian steroids stimulate opioid secretion from the hypothalamus (Wehrenberg et al., 1982b). Evidence that estradiol enhances the growth hormone response to clonidine via a stimulation of hypothalamic opioid secretion in humans has been presented (Phipps et al., 1989). In rodents, estradiol stimulates a population of hypothalamic opioid interneurons, which then stimulate GRF and inhibit SRIF cell activity within the hypothalamus (reviewed by Berthelier et al., 1989).

The ability of androgens to stimulate growth has been assessed in boys with delayed puberty (reviewed by Kerrigan and Rogol, 1992; Zachmann, 1992). The growth response to androgens is largely dependent on the presence of growth hormone. In growth hormone-sufficient individuals, androgen administration increased 24-hour growth hormone levels, growth hormone pulse amplitude, and circulating IGF-I levels (Parker et al., 1984). In growth hormone-deficient individuals, androgen administration failed to stimulate serum IGF-I levels. In this latter group, treatment with both androgen and growth hormone elevated serum IGF-I levels and stimulated growth.

It has been suggested that the aromatization of testosterone to estrogen in normal boys contributes significantly to pubertal growth. There are several lines of evidence to support this hypothesis. First, there is a positive correlation between estrogen levels and circulating growth hormone levels in boys. Growth hormone is higher in adult premenopausal women than in adult men or postmenopausal women, and growth hormone levels increase in men and postmenopausal women after estrogen administration (Wiedemann et al., 1976). Second, various androgens have been given to boys with pubertal delay to accelerate growth (reviewed by Zachmann, 1992). In such studies, the androgen oxandrolone was found to be less active than testosterone. Oxandrolone cannot undergo aromatization to an estrogen (Link et al., 1985; Ulloa-Aguirre et al., 1990), suggesting that the activity of testosterone is at least partially mediated by estrogenic metabolites. Neither oxandrolone nor dihydrotestosterone, another androgen incapable of aromatization, stimulated serum growth hormone or IGF-I levels in boys with delayed puberty (Keenan et al., 1993; Malhotra et al., 1993). Third, blockade of the androgen receptor with the androgen antagonist flutamide stimulated the growth hormone pulse height in pubertal boys (Metzger and Kerrigan, 1993). Finally, males with androgen insensitivity cannot respond to androgens because of expression of a defective androgen receptor. Estrogen administration to these individuals stimulates pubertal growth (Zachmann et al., 1986).

d. NUTRITIONAL STATUS AND GROWTH HORMONE SECRETION. Growth hormone plays an important physiological role in metabolic regulation. It promotes nitrogen retention and lipolysis and preserves serum glucose levels. Dietary metabolites modulate growth hormone secretion in a way that preserves lean body mass during periods of caloric restriction. In humans, the daytime pulsatile pattern of growth hormone secretion is masked by the effect of dietary metabolites. Pulsatile growth hormone secretion is quite evident following 1 to 5 days of fasting (Ho et al., 1988). The effects of glucose, the amino acid arginine, and free fatty acids on growth hormone secretion are particularly well studied. Intravenous infusion of insulin (0.1 units/kg) to induce hypoglycemia and arginine (0.5 g) are standard clinical measures used to assess pituitary growth hormone secretion in growth-deficient individuals. Oral glucose provokes an acute and marked suppression of growth hormone secretion that is followed by a delayed increase in plasma growth hormone 3 to 5 hours later (Valcavi et al., 1992). The immediate decrease in serum growth hormone levels is mediated by an acute release of somatostatin by glucose, and a rebound depression of so-

matostatin secretion is thought to explain the delayed increase in growth hormone. The effects of glucose are believed to be mediated by a "glucoreceptor" within the hypothalamus that stimulates somatostatin release. Acute elevations in somatostatin, following oral or intravenous glucose, are sufficient to blunt growth hormone secretion evoked by GRF (Masuda et al., 1985).

Insulin-induced hypoglycemia results in elevated plasma growth hormone levels secondary to a depression in somatostatin secretion. The effect of insulin on growth hormone secretion, like that of glucose, is probably mediated by a hypothalamic glucoreceptor that senses blood glucose levels. Growth hormone secretion in response to hypoglycemia is more pronounced in females than males and is enhanced in males by estrogen administration.

In addition to arginine, growth hormone secretion can also be evoked by the infusion of other amino acids and ingestion of a high protein meal. Growth hormone secretion in response to arginine is also enhanced by estrogens, consistent with the idea that arginine inhibits somatostatin secretion and estrogens stimulate GRF secretion.

Free fatty acids reduce growth hormone secretion in humans (Imaki et al., 1985). Infusion of free fatty acids inhibits growth hormone secretion in response to a variety of stimuli, including GRF, insulin, arginine, exercise, and L-DOPA. The effect of free fatty acids is probably mediated by a direct suppression of growth hormone secretion from the pituitary, but elevations in circulating somatostatin levels also may contribute.

e. GROWTH HORMONE CHANGES ASSOCIATED WITH AGING. The pituitary content of growth hormone increases with age and circulating levels of growth hormone decrease. The reduction in circulating growth hormone levels with increasing age is associated with a decrease in growth hormone pulse amplitude and no change in pulse frequency (Simpkins and Millard, 1987; Vermeulen, 1987). Declining serum growth hormone levels are correlated with a decrease in serum IGF-I levels and influence growth hormone responses that are IGF-I mediated, e. g., lean body mass, adipose tissue, and skinfold thickness (Florini et al., 1985; Rudman et al., 1990; Vermeulen, 1987). The magnitude of the change in serum growth hormone levels with age is more marked in women than men; this difference is explained by the decline in circulating estrogen levels that mark the onset of menopause (Ho et al., 1987). Age-associated changes in sleep patterns and body weight (obesity) were controlled for in these studies and do not appear to contribute to the decline in growth hormone. Similarly, testosterone levels do not show a significant decline with age in men and, therefore, are, most likely, not a factor in the observed reductions in serum growth hormone (Vermeulen, 1987). The possible role of reduced exercise in aging populations in the decline of serum growth hormone requires more thorough investigation. It has been reported, however, that the growth hormone response to resistance exercise in an older population of individuals (mean age, 72 years) was significantly diminished compared with that seen in a younger population (mean age, 27 years) (Pyka et al., 1992).

4. Regulation of growth hormone secretion by negative feedback. a. GROWTH HORMONE. Growth hormone has little or no direct effect on the pituitary gland in the inhibition of secretion in vitro. Growth hormone infusion in humans rapidly and completely inhibits GRF-stimulated release of growth hormone from the pituitary (Keijman and Frohman, 1991). Based on these results, it is hypothesized that growth hormone negative feedback is due to a stimulation of somatostatin release. Experiments in human subjects do not rule out the possibility that growth hormone may also decrease GRF output.

b. INSULIN-LIKE GROWTH FACTOR-I AND INSULIN-LIKE GROWTH FACTOR-II. The role of IGF-I and IGF-II in the regulation of growth hormone secretion has been investigated extensively in animals (reviewed by Casanueva, 1992; Muller, 1987). Several mechanisms have been implicated in IGF-I inhibition of growth hormone secretion in rodents: (*a*) inhibition of growth hormone gene transcription within the pituitary, (*b*) inhibition of growth hormone secretion from the pituitary, and (*c*) hypothalamic effects suppressing pulsatile growth hormone secretion from the pituitary. The first two mechanisms are mediated through IGF-I receptors on anterior pituitary cells. The role of IGF-I in the hypothalamus is controversial, but two recent studies using purified recombinant human IGF-I and IGF-II have shown that the presence of both IGF-I and IGF-II is necessary to exert a centrally mediated inhibition of growth hormone secretion in rodents in vivo (Harel and Tannenbaum, 1992a,b).

5. Regulation of growth hormone secretion by neurotransmitters. GH secretion from the pituitary is the net result of interacting neuroendocrine pathways. Neurons that control changes in somatostatin secretion from the hypothalamus are an important determinant of serum GH levels in humans. A "cholinergic" hypothesis of growth hormone regulation has been developed based on a large number of observations in humans (fig. 4) (reviewed by Casanueva, 1992; Devesa et al., 1992). In this model, central cholinergic tone modulates the activity of central α- and β-adrenergic nerves, which in turn dictate somatostatin, and possibly also GRF, secretion. Additionally, cholinergic activity may influence somatostatin secretion independently of effects on adrenergic pathways. Growth hormone secretion from the pituitary is determined by the relative amounts of GRF and somatostatin that reach the gland.

a. CHOLINERGIC PATHWAYS. Cholinergic pathways are involved in the regulation of growth hormone secretion stimulated by sleep, exercise, arginine, clonidine, and opioids in humans. All of these growth hormone responses are inhibited by muscarinic cholinergic antago-

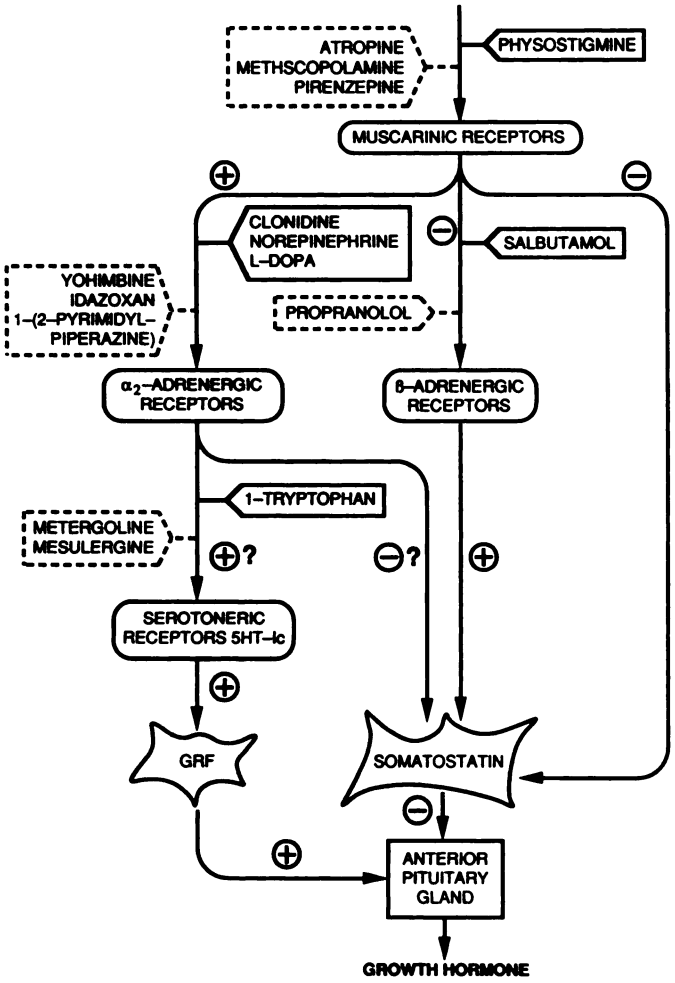

FIG. 4. Neurological/pharmacological control of growth hormone secretion. 5HT-1c, 5-hydroxytryptamine type 1c.

nists such as atropine, methscopalamine, and pirenzepine (Casanueva et al., 1984a; Massara et al., 1984). In rats and humans, muscarinic antagonists abolish growth hormone secretion in response to GRF (Locatelli et al., 1986). Two mechanisms have been proposed: (*a*) anticholinergic stimulation of somatostatin release within the hypothalamus or median eminence and (*b*) anticholinergic inhibition of GRF action on the pituitary gland. These mechanisms are not mutually exclusive, and both may be involved. In support of the first model, acetylcholine administration or enhancement of cholinergic tone with physostigmine inhibit somatostatin release and enhance the growth hormone secretory response to GRF (Kelijman and Frohman, 1991). However, evidence that anticholinergics exert their effects on the pituitary gland or the median eminence rather than within the central nervous system is provided by pirenzepine, a peripherally acting antimuscarinic agent (Massara et al., 1984).

b. ADRENERGIC PATHWAYS. Central adrenergic receptors affect plasma growth hormone levels in two ways. α_2-Adrenergic agonists stimulate the release of growth hormone, whereas β-adrenergic agonists inhibit growth hormone secretion (Ghigo et al., 1990). The α_2-adrenergic agonists, clonidine and L-DOPA, are growth hormone secretagogues used in clinical settings to stimulate growth hormone release. The α_2-adrenergic antagonist, yohimbine, blocks the growth hormone response to clonidine and L-DOPA (Devesa et al., 1990).

At the current time, the mechanism of the α_2-adrenergic stimulation of growth hormone secretion is unclear. Initial data suggested that clonidine and L-DOPA release GRF (reviewed by Camanni et al., 1989). Clonidine can enhance GRF-stimulated growth hormone secretion, however, and, therefore, may have actions independent of GRF release. It has been suggested that clonidine increases growth hormone levels by decreasing plasma levels of somatostatin, but there are no direct data to support this idea (Devesa et al., 1990; Penalva et al., 1993). Experiments with the synthetic GRF analog, GHRP-6, have provided evidence of a new, non-GRF receptor involved in growth hormone secretion in humans (Penalva et al., 1993). However, until an endogenous ligand is isolated, the true role of the growth hormone-releasing peptide remains obscure. Propranol, which does decrease the somatostatin level, but not clonidine, facilitates growth hormone release via GHRP-6, providing additional evidence that clonidine does not lower somatostatin levels (Penalva et al., 1993).

The possibility that clonidine's effects are mediated by serotonin release has been raised based on experiments in rodents (Conway et al., 1990). Aulakh and colleagues (1992) have proposed that α_2-adrenoceptors are located on serotonergic presynaptic nerve terminals and that activation of the α_2-receptors stimulates serotonin and, subsequently, GRF release. Clonidine stimulation of serum growth hormone levels in this report were completely blocked by the administration of either yohimbine, the α_2-adrenergic antagonist, or the 5-hydroxytryptamine type Ic receptor antagonist, mesulergine.

Not all models of growth hormone secretion include a role for serotonin in GRF release (Casanueva et al., 1984b). However, involvement of serotonin in growth hormone release mechanisms may explain the alterations in growth hormone secretion associated with human depression.

β-Adrenoceptors regulate growth hormone secretion by modulating somatostatin secretion (reviewed by Muller, 1987). Inhibition of hypothalamic somatostatin secretion by the β_1-adrenergic antagonist, propranolol, enhances the growth hormone response to hypoglycemia, exercise, glucagon, and GRF (Dieguez et al., 1988). In contrast, the β_2-adrenergic agonist, salbutamol, increases somatostatin secretion and decreases plasma growth hormone levels.

c. NEUROPEPTIDES. GRF and somatostatin are by far the most important regulators of growth hormone secretion. However, in rodents, several other neuropeptides influence growth hormone secretion from the pituitary gland. These include bombesin, endogenous opioids, gal-

anin, gastrin, neurotensin, substance P, thyroliberin, and vasoactive intestinal peptide (reviewed by Casanueva, 1992). Some of these peptides, including GRF and SRIF, are produced in the human pituitary gland (Joubert et al., 1992). Hence, in addition to the hypothalamic pituitary axis, some autocrine regulation of growth hormone secretion may take place. In humans, the role of endogenous opioids and galanin in the regulation of growth hormone secretion has been most extensively investigated in vivo.

Opioid stimulation of growth hormone secretion in humans may be mediated through the μ-opioid receptor (Giusti et al., 1992) and appears to be related to GRF responsiveness. Secretion of growth hormone in women treated with GRF is attenuated in the presence of naloxone (DeMarinis et al., 1991). In prepubertal children, there is evidence that circulating levels of β-endorphin enhance the responsiveness of the pituitary to GRF (Pugliese et al., 1992). In adult women, endogenous opioids may also mediate the elevation of serum growth hormone levels in response to estradiol (Wehrenberg et al., 1982b). Growth hormone secretion in response to opioids is blunted in elderly men (mean age, 76 years). This may be due to age-related reductions in the activity of cholinergic pathways, because cholinergic activity suppresses somatostatin secretion. Consequently, older individuals exhibit a blunted responsiveness to both opioids and GRF (Giusti et al., 1992).

Galanin is a 29-amino acid neuropeptide. Human galanin has a unique structure and exhibits species-specific differences in its biological effects (Evans and Shine, 1991). In humans, infusion of galanin enhances growth hormone secretion induced by GRF and arginine (Giustina et al., 1992b); the mechanism of this action of galanin is still unclear. Evidence consistent with the hypothesis that galanin acts at the levels of the hypothalamus (Ghigo et al., 1992; Hulting et al., 1991) and the pituitary gland (Giustina et al., 1992b; Hsu et al., 1991) exist. Experiments in rodents and humans suggest that the actions of galanin and GRF on growth hormone secretion are interrelated. In addition, estrogen has a permissive effect on galanin-induced growth hormone secretion in humans (Giustina et al., 1993).

D. Growth Hormone Target Tissues

Growth hormone regulates growth through hypertrophy, hyperplasia, or both, as a result of tissue differentiation, cell proliferation, and protein synthesis. Growth hormone can exert its effects either directly or through the actions of a mediator, IGF-I. IGF-I (somatomedin C) is a highly conserved 70-residue protein that is produced in many tissues (but primarily the liver) in response to growth hormone and other factors. IGF-I bears structural homology to insulin and to a related growth factor, IGF-II, and yet appears to act through its own unique receptor to enhance the proliferation and/or maturation of many tissues, including bone, cartilage, and skeletal muscle. IGF-I is suppressed or undetectable in the serum and tissues of growth hormone-deficient individuals but can be stimulated by growth hormone administration. Growth hormone stimulation of IGF-I expression can either occur in a paracrine manner at the target tissue or through a distal endocrine manner by IGF-I production at tissues such as the liver. The molecular mechanisms governing growth hormone regulation of IGF-I expression are not understood but appear to reside at the transcriptional level (Bichell et al., 1992). Growth hormone and IGF-I interact with many other hormones (including thyroxine, cortisol, and insulin) and growth factors (e.g., transforming growth factor-β, basic fibroblast growth factor) to modulate somatic growth.

Growth hormone also exerts metabolic effects on target tissues. Growth hormone can stimulate lipolysis and protein synthesis and modulate carbohydrate metabolism and fluid-electrolyte balance. The actions of growth hormone on carbohydrate metabolism are complex, having both acute and delayed effects. Acutely, growth hormone can stimulate insulin release from pancreatic β-cells, producing hyperinsulinism. Also, glucose oxidation is transiently enhanced in vitro by growth hormone but is suppressed within 1 hour. The delayed effects of growth hormone are antagonistic to those of insulin ("diabetogenic effect"). This observation is supported by the fact that patients with acromegaly often have mild to moderate hyperglycemia and insulin resistance.

1. Bone and Cartilage. Historically, growth hormone effects on bone and cartilage were initially recognized in clinical states of growth hormone excess and deficiency. Growth hormone deficiency results in failure of somatic growth in the postnatal period. Of the several hormones and growth factors that modulate skeletal growth, only growth hormone appears capable of stimulating longitudinal bone growth (Isaksson, et al., 1987). Longitudinal bone growth occurs at the epiphyseal growth plate, a structure of complex histology, that provides a template for bone formation prior to puberty. The epiphyseal plate, which is located at the proximal and distal ends of long bones, is composed of a prechondrocyte germinal cell layer that can differentiate into chondrocytes in adjacent areas of the growth plate, termed the proliferative zone and hypertrophic cell layer (Howell and Dean, 1992).

Growth hormone was previously thought to modulate somatic growth solely through the production of IGF-I at distal sites such as the liver, but in vivo studies in rats suggest that local infusion of growth hormone into the epiphyseal plates of hypophysectomized rats produces unilateral growth at the site of infusion, suggesting either a direct effect or local production of IGF-I in the epiphyseal plate (Isaksson et al., 1987). Previous evidence suggests that growth hormone modulates somatic growth at the epiphyseal plate by inducing differentiation and pro-

liferation of prechondrocytes in the germinal cell layer (Ohlsson et al., 1992). Whether these effects are the direct actions of growth hormone or the paracrine effects of IGF-I at the epiphyseal plate is unclear, but recent studies suggest that growth hormone is capable of stimulating the multiplication of germinal cells, and IGF-I is capable of acting on the proliferation of resulting chondrocytes (Ohlsson et al., 1992). This observation corroborates the "dual effector theory" put forth by Green and coworkers (1985), which states that growth hormone and IGF-I act through separate but synergistic mechanisms to modulate somatic growth.

Growth hormone may promote differentiation of precursor cells to become responsive to IGF-I produced either locally within the target tissue or distally by hepatic production. In addition to effects on longitudinal bone growth, growth hormone also appears to exert modest effects on bone mineral density, particularly in adults with isolated growth hormone deficiency (O'Halloran et al., 1993).

2. Adipose Tissue. One site where growth hormone modulates differentiation and growth is adipose tissue. The effects of growth hormone on tissue differentiation have been examined in vitro. Several "preadipocyte" (fibroblastoid) cell lines exist that can be induced to undergo differentiation to adipocytes by growth hormone (3T3-F442A, 3T3-L1, and Ob 1771 cell lines) (Hauner, 1992). This effect is specific for growth hormone and cannot be induced by IGF-I. As these cells are induced to differentiate into cells having adipocyte morphology, a series of transcriptional events coincides with differentiation, which includes expression of the early response genes within minutes to hours, followed by transcriptional activation of genes that coincide with terminal differentiation of the cells into adipocytes (several hours to days). The metabolic effects of growth hormone on lipolysis become more evident as these cells develop the adipocyte phenotype. In children and adults, growth hormone decreases fat deposits and increases lean body mass. Serum levels of free fatty acids and glycerol increase within 4 hours of growth hormone administration to normal adults.

3. Liver. Growth hormone regulates the expression of a variety of proteins in the liver (table 2). In rats, cytochrome P450 2C11 and 2C12 are induced after several days of growth hormone treatment. The regulation of P450 isoenzyme expression by growth hormone has been studied extensively in rats and displays sex-dependent patterns of gene expression (Al-Shawi et al., 1992; Levgraverend et al., 1992; Sundseth et al. 1992; Sundseth and Waxman, 1992).

TABLE 2
Growth hormone-regulated genes

Rapid transcriptional activation
c-*fos*
c-*jun*
IGF-I
Serine protease inhibitor
Intermediate transcriptional activation
Lipoprotein lipase (via c-*fos*)
Slow/subacute transcriptional activation
Cytochrome P450 SC11, SC12
α_2-Microglobulin

The role of growth hormone in the regulation of hepatic biosynthesis of lipoproteins and lipoprotein receptors has been demonstrated in several recent clinical studies. Hepatic LDL receptors were increased 2- to 3-fold in elderly adults following the administration of 2 subcutaneous injections of 12 IU of growth hormone (Rudling et al., 1992). Hepatic LDL receptors take care of the removal of cholesterol from the circulation, and individuals treated with human growth hormone exhibited a statistically significant reduction in circulating levels of LDL (Eden et al., 1993; Olivecrona et al., 1993). The increase in hepatic LDL receptors may mediate the cholesterol-lowering effect of growth hormone reported by some investigators (Binnerts et al., 1992; Olivecrona et al., 1993; Salomon et al., 1989). Growth hormone either increases (Eden et al., 1993) or has no effect (Olivecrona et al., 1993) on high-density lipoprotein levels in humans.

In contrast to the favorable effects of growth hormone on plasma lipoprotein profiles with regard to cardiovascular disease that were discussed above, growth hormone stimulates hepatic production of lipoprotein (a) (Olivecrona et al., 1993; Eden et al., 1993). Increased lipoprotein (a) levels are associated with increased risk of cardiovascular disease. In addition, one group of investigators found that growth hormone administration elevated serum triglyceride levels in older patients (Friedman et al., 1972, 1974). It appears, therefore, that growth hormone is an important physiological regulator of hepatic lipoprotein biosynthesis in humans.

4. Immune system. The study of the action of growth hormone on the human immune system is an area of active investigation (reviewed by Gelato, 1993). Human lymphocytes express growth hormone receptors and synthesize the growth hormone protein (Gala, 1991; Weigent et al., 1988). Growth hormone elicits a wide variety of immunological responses when added to lymphocytes in vitro (Kelley, 1989). Both growth hormone and IGF-I stimulate cell proliferation in primary lymphoid tissue (e.g., bone marrow, thymus) and peripheral macrophages and lymphocytes in rats (Gelato, 1993). In humans, most evidence suggests an effect on the T-cell population (Crist and Kraner, 1990; Geffner et al., 1990); however, growth hormone-deficient patients do not exhibit gross deficiencies in immunological responsiveness (Spadoni et al., 1991).

5. Reproductive system. Growth hormone plays an important role in normal human reproduction (reviewed by Katz et al., 1993). Puberty is delayed in growth hormone-deficient children and is stimulated by growth hormone replacement. Growth hormone is required for gonadal

development in girls and boys (reviewed by Cristman and Halme, 1992; Spiteri-Grech and Nieschlag, 1992). The mechanism of action of growth hormone is to sensitize the ovary to the effects of gonadotropins. In rodents, there is evidence that this occurs via growth hormone-induced increases in luteinizing hormone receptor levels. These effects of growth hormone on the ovaries and testis may be the result of increased circulating levels of IGF-I or local production of IGF-I. In addition, tissue culture studies have shown that growth hormone can enhance progesterone synthesis in human luteal cells stimulated with human chorionic gonadotropin and estradiol synthesis in human granulosa cells stimulated with follicle-stimulating hormone (Lanzone et al., 1992; Mason et al., 1990).

E. Alterations in Growth Hormone Secretion

Changes in growth hormone secretion patterns occur in several physiological states including obesity, diabetes, starvation, and acromegaly. In many abnormal states, growth hormone pulse frequency rather than pulse amplitude is altered. In addition, pregnancy results in the elevated, nonpulsatile expression of *GH-V*.

1. Obesity. The decrease in growth hormone secretion observed in obese individuals is the result of a 3-fold decrease in pulse frequency and an increased clearance of growth hormone from the circulation (Veldhuis et al., 1991). Obese individuals exhibit a blunted growth hormone secretory response to a wide variety of stimuli including insulin, arginine, opiates, glucagon, L-DOPA, sleep, and GRF, suggesting the presence of high circulating somatostatin levels.

2. Diabetes/starvation. Growth hormone levels are elevated 2- to 3-fold during starvation and in diabetes (Asplin et al., 1989; Ho et al., 1988; Pimstone et al., 1966). Increased growth hormone pulse frequency is the major factor contributing to increased 24-hour growth hormone secretion under both circumstances. Elevated growth hormone is one of several factors proposed to play a role in the microvascular complications of diabetes (Holly et al., 1988). Elevated serum glucose levels in diabetic patients fail to suppress growth hormone secretion, as occurs in normal individuals (Press et al., 1984). Furthermore, normalization of serum glucose levels with insulin and diet failed to normalize serum growth hormone levels (Miller et al., 1992).

The neuroendocrine basis for the abnormalities in growth hormone secretion in patients with diabetes and starvation is unknown. However, in both diabetes and starvation, serum IGF-I levels are reduced, indicating that the normal coupling of serum growth hormone with IGF-I secretion from the liver is disrupted.

Anorexia nervosa is a psychological disorder resulting in malnutrition. Patients have reduced serum estradiol levels and elevated growth hormone levels. As seen during starvation and diabetes, levels of IGF-I in patients with anorexia nervosa are paradoxically low (Dieguez et al., 1988).

3. Acromegaly. The abnormally high circulating levels of growth hormone seen in patients with acromegaly are associated with high-frequency release of GRF that is unresponsive to negative feedback inhibition by IGF-I (Ho et al., 1992). Repeated injection of GRF in acromegalic patients also resulted in continued growth hormone secretion. Somatostatin insensitivity secondary to a down-regulation of somatostatin receptors by chronically elevated growth hormone and/or somatostatin has also been proposed as a mechanism contributing to elevated growth hormone secretion in acromegalics (Brazeau, 1990).

4. Hyper-/hypocortisolism. Normal cortisol levels participate in the maintenance of normal amounts of serum growth hormone, because adrenocorticotrophic hormone-deficient individuals require glucocorticoid-replacement therapy to restore normal levels of growth hormone (Giustina et al., 1989). However, chronic elevations in serum cortisol concentrations, as occur in patients with Cushing's disease or patients treated with glucocorticoids for immunosuppression, result in a decrease in plasma growth hormone levels (Giustina et al., 1992a). In young individuals, the decrease in serum growth hormone results in reduced somatic growth (Blodgett et al., 1956). Elevated serum glucocorticoid levels impair the growth hormone secretory response to a variety of agents including clonidine, GRF, arginine, and insulin (Giustina et al., 1992a; Nakagawa et al., 1969). These data are consistent with the hypothesis that chronically elevated glucocorticoid levels reduce circulating levels of growth hormone by increasing somatostatin secretion (reviewed by Giustina and Wehrenberg, 1992).

Dexamethasone, a synthetic glucocorticoid, increases the activity of peripheral β-adrenergic pathways by increasing β-adrenoceptor levels (Collins et al., 1991). It has been postulated that a similar effect of glucocorticoids on central β-adrenergic pathways stimulates somatostatin secretion from the hypothalamus (Lima et al., 1993). This hypothesis is supported by the observation that propranolol, a β-adrenoceptor antagonist, overcomes the effect of dexamethasone on growth hormone levels.

Three hours following injection of glucocorticoids, serum growth hormone levels are elevated (Casanueva et al., 1990). The stimulation of growth hormone and GRF receptor synthesis by glucocorticoids may be involved in this acute response (Seifert et al., 1985; Strobl et al., 1989).

5. Hypothyroidism. Delayed growth is observed in children with hypothyroidism. Although basal serum growth hormone levels are frequently within normal range, many hypothyroid children exhibit a blunted growth hormone secretory response to hypoglycemia and/or arginine and have lower than normal IGF-I levels (Chernausek et al.,

1983; MacGillivray et al., 1968). In adult hypothyroidism, circulating levels of IGF-I are low, and there may be a reduced growth hormone secretory response to hypoglycemia (Chernausek et al., 1983). Growth hormone secretory responses and IGF-I levels are restored to normal by thyroid hormone replacement therapy.

In rodents, hypothyroidism is associated with a complete loss of pulsatile growth hormone secretion and a decreased growth hormone synthesis by the pituitary (Katakami et al., 1986).

6. Pregnancy. Growth hormone levels in the maternal and fetal circulation are elevated during pregnancy (reviewed by Hill, 1992). During the first 22 weeks of gestation, growth hormone is synthesized in the maternal pituitary by the expression of the *GH-N* gene. From 22 weeks' gestation to parturition, serum levels of growth hormone in the mother continue to increase; the increase in serum growth hormone during the latter half of pregnancy is due to expression of the *GH-V* gene in the placenta. Growth hormone expression by the placenta is nonpulsatile and does not respond to GRF. Growth hormone is also produced in the fetal pituitary by 12 weeks' gestation and reaches a maximum level at 22 to 27 weeks' gestation. Levels of growth hormone circulating in the fetus at this time are elevated approximately 4-fold compared with a normal child.

The role of growth hormone in pregnancy is under investigation. Placental lactogen, prolactin, and growth hormone stimulate pancreatic β-cell proliferation. This may facilitate production of insulin during pregnancy (Brelje et al., 1993). Circulating IGF-I levels are elevated in response to growth hormone. Many studies indicate that fetal growth is primarily determined by placental lactogen (Eriksson et al., 1989a,b; Kaplan and Grumbach, 1967; Seekal, 1960). However, growth hormone and growth hormone receptors are present in the skin, growth plate chondrocytes, and osteoclasts in human fetuses at 15 to 20 weeks' gestation (Werther et al., 1993). These data suggest that growth hormone does play a role in human fetal growth.

IV. Therapeutic Uses of Human Growth Hormone

A. Pharmacology of Recombinant Human Growth Hormone

1. Preparations. The commercially available preparations of human growth hormone are summarized in table 3. Two preparations produced in bacteria through recombinant DNA technology are approved for human use in the United States. Protropin (Genentech, San Francisco, CA) is methionyl human growth hormone and differs from the native human hormone by the addition of one amino acid, methionine, at the amino terminus. Humatrope (Eli Lilly, Indianapolis, IN) is authentic human growth hormone produced in bacteria. Kabi-Vitrum AB, Stockholm, Sweden, is a European supplier of recombinant methionyl human growth hormone (Somatonorm) and authentic human growth hormone (Genotropin) (Wilton et al., 1987). Norditropin is authentic recombinant human growth hormone produced by Novo-Norsk A/B, Denmark. Human growth hormone has also been produced in mammalian tissue culture cells for therapeutic use (Saizen, Serono GmbH, Freiburg, Germany) (Pavlakis and Hamer, 1983; Zeisel et al., 1992b). All of the recombinant human growth hormone preparations and pituitary-derived human growth hormone have equivalent therapeutic efficacies and pharmacokinetic properties (Moore et al., 1988; Pavia et al., 1992; Refetoff and Sonksen, 1970; Stubbe et al., 1992; Takano et al., 1987; Zeisel et al., 1992b).

TABLE 3
Human growth hormone preparations

Natural sequence
Humatrope
Genotropin
Saizen
Methionyl-human growth hormone
Protropin
Somatonorm
Norditropin

Standardization of human growth hormone dosage regimens would facilitate comparisons among different studies. At the present time, growth hormone doses have been expressed as mg/kg/day or week and as IU/kg or IU/m^2 body surface area/day or per week or per year. One IU = 2.7 mg of human growth hormone. Because of the widespread use of purified recombinant human growth hormone, the use of mg dosages rather than IU is appropriate (Rosenfeld et al., 1990).

Some studies indicate that there is a higher incidence of growth hormone antibody production in individuals treated with methionyl human growth hormone than with authentic human growth hormone (Takano et al., 1987; Tyllstrom et al., 1985). The greater antigenicity of the methionyl form has been linked to trace contamination of methionyl human growth hormone with bacterial proteins rather than because of the presence of the extra methionine group. This problem has since been alleviated by improved purification. Antibody production rarely interferes with the biological activity of human growth hormone because the antibodies are low in affinity and concentration (Takano et al., 1987). Furthermore, growth hormone antibody levels decrease following discontinuation of methionyl-human growth hormone (Massa et al., 1993). Mammalian cell-derived human growth hormone has very low immunogenicity (Zeisel et al., 1992a).

2. Pharmacokinetics. All growth hormone preparations are delivered by injection. Intramuscular and subcutaneous routes are equally effective (Russo and Moore, 1982). Subcutaneous injection is preferable because it is less painful. Serum growth hormone levels are greater following subcutaneous abdominal injection than subcutaneous thigh injection; however, equivalent stimula-

tion of IGF-I levels are seen (Beshyah et al., 1991). Absorption is delayed following all routes of injection with peak plasma levels of growth hormone occurring 3 to 5 hours after injection (reviewed by Kearns et al., 1991). There is considerable interindividual variation in absorption rates.

Noninjectable routes of growth hormone administration are under investigation. Ron et al. (1993) have developed a controlled release system for long-term growth hormone administration. These investigators incorporated bovine growth hormone into a biodegradable polyanhydride polymer that was implanted into the lower abdominal region of rats. Biologically active growth hormone was released into the circulation for 3 weeks. Serum levels of growth hormone have been achieved after administration of growth hormone to humans intranasally. To facilitate absorption, growth hormone was given in combination with 1% sodium tauro-24,25-dihyrofusidate, a membrane-permeabilizing agent (Hedin et al., 1993). No adverse effects were reported. A single 0.27-mg/kg dose of growth hormone was absorbed 1.5 to 3% as efficiently by the intranasal route as compared with the subcutaneous injection. Serum levels 15% of those seen following a 0.27-mg/kg subcutaneous injection were achieved by increasing the dose of growth hormone administered intranasally to 10.8 mg/kg. Thus, intranasal administration of growth hormone represents a feasible and more convenient, albeit more expensive, drug delivery route. It is unclear, however, based on the data presented by Hedin et al., 1993, whether the membrane-permeabilizing agent aided growth hormone absorption.

In the circulation, growth hormone is extensively bound to a high-affinity growth hormone-binding protein. Binding to this protein extends the half-life of growth hormone in the circulation. The time of day (e.g., morning versus evening) does not influence the serum levels of growth hormone achieved (Jorgensen et al., 1990); evening administration mimics the physiological peak of serum growth hormone seen with the onset of slow-wave sleep.

The growth hormone elimination half-life has been estimated to be between 2 and 3 hours. Elimination is primarily via metabolism in the liver and kidney and is more rapid in adults than in children (Kearns et al., 1991). Little intact growth hormone is excreted in the urine (Sohmiya and Kato, 1992).

Growth hormone administration has the potential for drug interactions. Growth hormone reduces the activity of type I and type II mixed function oxidase enzymes in the liver (Eriksson et al., 1989b; Levitsky et al., 1989; Redmond et al., 1980).

B. Treatment of Short Stature in Children and Adolescents

1. Isolated growth hormone deficiency. Isolated growth hormone deficiency is the clearest indication for replacement therapy with human growth hormone (reviewed by Frasier and Lippe, 1990; Laron and Butenandt, 1991; Lippe and Nakamoto, 1993; Wilson, 1992). The clinical signs are growth retardation as evidenced by height 2SD below the mean predicted on the basis of age and gender. The diagnosis is confirmed by the finding of a deficit in growth hormone secretion (<4 to 7 ng/ml) in response to one or more provocative stimuli (e.g., clonidine, arginine, insulin, L-DOPA). The efficacy of human growth hormone preparations has been evaluated most thoroughly in this patient population; no serious side effects of replacement therapy have been observed to date. Early fears that growth hormone replacement would result in glucose intolerance have not been confirmed. Furthermore, growth hormone replacement in children improves bone density (Saggese et al., 1993) and has a favorable effect on lipid profiles, i.e., there is a lowering of serum cholesterol and LDL levels (De Muinck Keizer-Schrama et al., 1992).

The nature of the growth hormone preparation (methionyl human growth hormone versus authentic human growth hormone) is not a factor in growth stimulation. Dosage frequency is an important determinant of treatment response. For a given total weekly dose of human growth hormone, the growth response (height velocity) is greater with more frequent administration (Albertsson-Wikland and Rosberg, 1987). Daily subcutaneous administration is recommended because it is more effective than the three times/week or once weekly injection schedules used previously (Flodh, 1987; Kastrup, et al., 1983; Kearns et al. 1991; Kikuchi et al., 1988).

Although dose-response relationships have been reported with growth hormone replacement therapy, many factors influence the magnitude of the growth response (reviewed by Ranke and Blank, 1990; Sherman, 1987). The strongest dose-response relationship is seen in prepubertal children during their first year of treatment. Pretreatment height velocities in this group of individuals averages 4 cm/year or less, whereas during the initial year of treatment it increases to 9 to 12 cm/year at doses of 0.3 mg/kg/week, given in three divided doses, or 12 IU/m^2/week with daily injection. In general, growth responses are the greatest in patients with the slowest pretreatment growth rates.

Growth responses to replacement therapy are maximal during the initial 1 to 2 years of treatment. Height velocity decreases progressively during subsequent years of treatment and more rapidly in some individuals than others. Treatment is generally continued if the yearly growth rate exceeds the untreated growth rate by 2 cm/year. The reason for the decline in growth velocity is unknown, although in very rare instances it can be attributed to formation of growth hormone antibodies (De Muinck Keizer-Schrama et al., 1992; Wit et al., 1989).

Greater growth rates are achieved with growth hor-

mone replacement in young children (<10 years) than in adolescents (10 to 19 years). Dose-response relationships are not well established for pubertal children (Job et al., 1987). Hence, the earlier the diagnosis of isolated growth hormone deficiency is made, the more effective the treatment.

2. Panhypopituitarism. Panhypopituitarism is a congenital defect characterized by deficits in growth hormone, prolactin, adrenocorticotrophic hormone, gonadotropin, and thyroid hormone secretion. Growth hormone replacement is one aspect of growth management in these patients who will also require glucocorticoid and thyroid hormone replacement to stimulate childhood growth and gonadotropin (or estrogen/androgen replacement) to stimulate growth during puberty.

3. Postcranial irradiation. Defects in growth hormone secretion occur frequently in children secondarily to cranial irradiation for the treatment of leukemia and brain tumors (Ahmed et al., 1985; Chrousos et al., 1982; Kanev et al., 1991). This can be detected early following radiation by a pituitary failure to respond to growth hormone secretagogues; a 2-year course of treatment with replacement doses of human growth hormone is recommended in prepupertal children (Richards et al., 1991).

4. Turner's syndrome. Turner's syndrome is a genetic disease of females resulting from monosomy in the X chromosome or X chromosome mosaicism (Palmer and Reichmann, 1976). Turner's syndrome is characterized by low birth size, short adult height, obesity, and incomplete development of the ovaries, leading to low serum estradiol levels and poor expression of secondary sex characteristics (Rappaport and Sauvion, 1989). The growth deficit in patients with Turner's syndrome is partially due to subnormal estradiol levels. Estradiol is critical to the pubertal growth spurt and facilitates pulsatile growth hormone secretion in girls. Ethinylestradiol has been used successfully to stimulate growth during puberty in patients with Turner's syndrome (reviewed by Rappaport and Sauvion, 1989).

Growth hormone is not absent in patients with Turner's syndrome, but many studies have identified abnormalities in hormone secretion, including low 24-hour integrated growth hormone concentrations in serum, decreased nocturnal hormone secretion, and blunted growth hormone secretory responses to provocative stimuli (Brook, 1978; Butenandt, 1980; Rappaport and Sauvion, 1989; Zadik et al., 1992a). Serum IGF-I levels are reduced in prepubertal girls with Turner's syndrome (Ross et al., 1985). These data provide a rationale for treating patients with Turner's syndrome with human growth hormone, and a large number of studies indicate that growth is stimulated in patients with Turner's syndrome by growth hormone administration (reviewed by Rosenfeld, 1989).

The response of patients with Turner's syndrome to growth hormone differs from that seen in growth hormone-deficient individuals. Pharmacological doses of growth hormone are needed to stimulate growth (2.7 to 4.0 mg/kg/week) as compared with the replacement levels used in growth hormone deficiency (0.8 to 1.6 mg/kg/wk). Furthermore, the maximal growth velocity achieved by growth hormone treatment of patients with Turner's syndrome is less than occurs in growth hormone-deficient individuals. These results suggest that there may be some end-organ resistance to growth hormone and/or IGF-I in patients with Turner's syndrome.

Growth stimulation by the combination of human growth hormone and the synthetic androgen oxandrolone is synergistic and is the recommended treatment for growth stimulation in patients with Turner's syndrome during puberty (Rosenfeld, 1989; Rosenfeld et al., 1992). Replacement estradiol is given to adolescents with Turner's syndrome to stimulate secondary sex characteristic development during puberty but, surprisingly, is not as effective in combination with human growth hormone as is oxandrolone (Rosenfeld, 1989).

5. Down's syndrome. Down's syndrome is a genetic disease caused by trisomy of chromosome 21. Mental retardation, microcephaly, growth retardation, and congenital heart disease are characteristic features of patients with Down's syndrome. These individuals are small at birth and exhibit a depressed rate of growth throughout childhood and puberty. Castells et al. (1992) identified a growth hormone secretory defect in all individuals in a group of 20 noninstitutionalized patients with Down's syndrome. Thirty-five percent of the patients had subnormal growth hormone secretion in response to both clonidine and L-DOPA, and 65% failed to respond to one or the other stimulatory test. Serum IGF-I levels in all patients were in the low normal range, and all patients were euthyroid.

In a 1-year trial of methionyl human growth hormone (0.3 mg/kg/week, given in three divided doses), linear growth velocity in 13 patients with Down's syndrome was increased approximately 2-fold. Head growth was also stimulated (Torrado et al., 1991). The data available from this limited number of patients indicate that human growth hormone replacement is effective in the stimulation of growth in children with Down's syndrome. However, the use of human growth hormone in patients with Down's syndrome has been questioned on the ethical basis that increased stature will not result in an improved quality of life for these individuals (Allen, 1992).

6. Intrauterine growth retardation. Intrauterine growth retardation is characterized by a low birth weight and may also be associated with additional abnormalities (Silver-Russel syndrome). Children who do not exhibit catch-up growth during their first year of life have short adult stature. Some, but not all, studies show that growth hormone administration can stimulate catch-up growth in those children who do not spontaneously exhibit ac-

celerated growth (reviewed by Frasier and Lippe, 1990; Hindmarsh et al., 1991; Laron and Butenandt, 1991).

7. Idiopathic growth deficiency. Growth is determined by hormonal and nutritional factors. Well-nourished, slowly growing children who are below the fifth percentile for height in the growth curve for age and gender have been classified in different studies as having "normal variant short stature," "familial short stature," or "neurosecretory dysfunction" (reviewed by Frasier and Lippe, 1990; Hindmarsh et al., 1991; Wilson, 1992; Wit et al., 1989). None of these children meet the classic criteria for growth hormone deficiency but are clearly growing at subaverage rates. Children with normal variant short stature and familial short stature comprise a heterogeneous mixture with respect to growth hormone secretory activity. The underlying causes of normal variant short stature and familial short stature include neurosecretory defects and/or involvement of as yet undefined genetic factors. Among this group are individuals who exhibit lower than average 24-hour growth hormone concentrations, lower nocturnal growth hormone pulse amplitudes (with no change in pulse frequency), subnormal responses to one or more growth hormone secretagogues, and, most recently, low levels of growth hormone-binding protein (Albertsson-Wikland and Rosberg, 1987; Bercu, 1987; Fontoura et al., 1992; Takano et al., 1987; Zadik, et al., 1992c).

Children with idiopathic growth deficiency have been treated with replacement doses of human growth hormone similar to those used in the treatment of classic growth hormone deficiency (0.14 to 0.8 mg/kg/day). Growth velocity has significantly increased in 75 to 80% of these children (Lesage et al., 1991; Moore et al., 1992; Wit et al., 1989). The general use of human growth hormone in the treatment of idiopathic growth deficiency has not been approved in the United States, however. Several issues need to be resolved before growth hormone is more widely used.

The magnitude of the growth response is reduced compared to classic growth hormone-deficient children. It is possible that this could be improved by dosage optimization. It has been generally observed that children with the lowest initial growth velocities respond the best to growth hormone administration (Zadik, et al., 1992b,c), but additional criteria are needed to predict with certainty those children who will respond to treatment. Furthermore, the study data available to date do not allow a determination of the effects of growth hormone treatment on final adult height.

There are important scientific and ethical concerns regarding the general approval of human growth hormone for idiopathic growth deficiency. Fifty percent of all individuals fall below the "average" height, and there is no scientific basis for selecting who among these should receive growth hormone. In the extreme, widespread use of human growth hormone would only serve to increase the average height in successive generations. Finally, the potential for adverse side effects in children has not been thoroughly explored. Of greatest concern are controversial data associating the use of growth hormone in children with an increased risk of developing leukemia (reviewed by Wilson, 1992). Short stature may influence the development of self-esteem, but this does not justify the use of a treatment with an increased risk of cancer. The high cost of growth hormone therapy and the negative psychological effects produced if expected growth results are not achieved are additional ethical concerns (Allen and Fost, 1991; Schoen, 1991; reviewed by Hindmarsh et al., 1991; Laron and Butenandt, 1991; Stabler and Underwood, 1986).

8. Chronic renal failure. Children with chronic renal insufficiency grow slowly (Kleinknecht et al., 1983). Normal growth rates are not restored in these children by hemodialysis, although renal transplantation has a transient growth stimulatory effect (Kleinknecht et al., 1980). In most children, serum IGF-I levels are in the normal range, and responses to growth hormone secretagogues are adequate.

Growth velocity accelerated in prepubertal children with chronic renal insufficiency during human growth hormone replacement therapy. The magnitude of this effect was equal to that seen in growth hormone-deficient individuals (Anderson et al., 1992; Van Dop et al., 1992; Van Es, 1991). Pubertal children exhibited significant growth acceleration, but it was less than that observed in the prepubertal group (Van Es, 1991). As in isolated growth hormone deficiency and in idiopathic growth hormone deficiency, the beneficial effects of growth hormone therapy on growth velocity in children with renal insufficiency are most dramatic during the first year of therapy with lesser, but still significant, effects seen during the second year of treatment.

There is concern that the use of growth hormone in children with renal insufficiency may cause deterioration of renal function and rejection of renal transplants. Investigators have reported an increase in serum creatinine in patients treated with human growth hormone (Van Dop et al., 1992; Van Es, 1991). There are also reports of renal transplant rejection among recipients treated with human growth hormone (Anderson et al., 1992; Tyden et al., 1990; Van Es, 1991); however, Tnshoff et al. (1993) found no increased incidence of graft rejection during a 3-year study period. Finally, it has been suggested that the risk of cancer from growth hormone administration could be increased in these children due to the immunosuppressant therapy for graft retention (Hindmarsh et al., 1991).

9. Achondroplasia. The chondroplasias are a group of diseases characterized by defects in skeletal growth or where the growth axis is faulty. Growth hormone and serum IGF-I levels are normal, and the effects of supplemental growth hormone have not been explored exten-

sively in this patient population. A possible exception is achondroplasia in which preliminary, short-term therapy with human growth hormone has been shown to stimulate growth in some very slowly growing children (Hindmarsh et al., 1991; Horton et al., 1992; Laron and Butenandt, 1991; Nishi et al., 1993).

C. Use of Human Growth Hormone in Adults

Growth hormone synthesis and secretion continue throughout the life of a normal individual, but until recently, the physiological actions of growth hormone in adults were ill-defined. Individuals who were growth hormone deficient from childhood or had a deficiency secondary to an incident in adulthood were perceived as having adequate health, although increased body fat and decreased muscle strength were evident. Growth hormone-deficient adults show a predisposition for cardiovascular disease (Rosen and Bengtsson, 1990).

It is of interest to determine whether administration of growth hormone to growth hormone-deficient adults would be beneficial. The effects of human growth hormone have now been investigated in a limited number of studies among several adult populations: (*a*) growth hormone-deficient adults who had received replacement growth hormone during childhood (Binnerts et al., 1992; Jorgensen et al., 1991; Merimee et al., 1972; O'Halloran et al., 1993), (*b*) growth hormone-deficient adults with adult onset growth hormone deficiency (Bengtsson et al., 1993; Binnerts et al., 1992; Cuneo et al., 1991a,b; Degerblad et al., 1992; Salomon et al., 1989), (*c*) elderly normal adults (Aloia et al., 1976; Rudman et al., 1990), and (*d*) young normal adults (Crist et al., 1991; Deyssig et al., 1993; Friedman et al., 1972, 1974; Moller et al., 1991; Yarasheski et al., 1992).

In the first category are adults with isolated growth hormone deficiency in whom the diagnosis and treatment occurred during early childhood. Growth hormone therapy was discontinued in childhood when adequate height gains were achieved or when growth velocity was no longer increased by the treatment. In the second group are adults who underwent hypophysectomy, primarily for the treatment of a pituitary tumor. This group differs from category 1 in that growth hormone deficiency is accompanied by loss of thyroid hormone, adrenocorticotrophic hormone, and gonadotropins. Replacement therapy with thyroid hormone and thyroxine, glucocorticoids, and gonadal steroids is standard in these patients. Category 3, elderly adults 60 years of age or older, is a population that is relatively growth hormone deficient.

Growth hormone effects on body composition and metabolism in all adults with growth hormone deficits (categories 1, 2, and 3) are similar (reviewed by Lamberts et al., 1992). In section VI.C.1, the effects of growth hormone treatment reported in these studies are summarized according to target tissue. In section VI.C.2, the metabolic effects of growth hormone in normal young adults are reviewed. Sections VI.C.3 and VI.C.4 are devoted to the potential therapeutic usefulness of growth hormone in the treatment of infertility and the catabolic state.

1. Metabolic effects in adults with growth hormone deficits. a. ADIPOSE TISSUE. Growth hormone promotes lipolysis, and a statistically significant decrease in total body adipose tissue has been consistently observed in adults receiving human growth hormone. In growth hormone-deficient adults, the average decrease in body fat in three independent 6-month trials with human growth hormone ranged from 14 to 20% (Bengtsson et al., 1993; Rudman et al., 1990; Salomon et al., 1989). Adipose tissue associated with internal organs was reduced to a greater extent than subcutaneous tissue (Jorgensen et al., 1991).

b. MUSCLE. Lean body mass is significantly increased (5 to 11%) in growth hormone-deficient adults treated with human growth hormone and is associated with increased muscle mass and exercise capacity (Bengtsson et al., 1993; Cuneo et al., 1991a,b; Rudman et al., 1990; Salomon et al., 1989). It has been suggested that cardiac changes are responsible for the increased exercise capacity. Cardiac contractility and left ventricular cardiac mass are increased following growth hormone replacement therapy (Cuneo et al., 1991c; Thuesen et al., 1988). The role of increased muscle mass in exercise performance is unclear. Some investigators have found that increased muscle mass in the thigh and quadriceps was not accompanied by increased strength in these muscles (Cuneo et al., 1991a,b). However, in a study in which growth hormone-deficient adults received growth hormone for 16 months, muscle mass, exercise capacity, and muscle strength were all significantly increased. Despite the observed improvements, the growth hormone-deficient adults treated for 16 months with growth hormone did not achieve the exercise performance seen in normal age-matched subjects (Jorgensen et al., 1991).

c. BODY WATER. Growth hormone treatment in adults increases total body extracellular fluid (Pratt et al., 1993). This is manifested as an acute increase in body weight, swollen fingers and ankles, joint arthralgias, and carpal tunnel syndrome (Bengtsson et al., 1993; Binnerts et al., 1992; Moller et al., 1991; Salomon et al., 1989). The antinatriuretic effect of growth hormone is mediated in part by the activation of the renin-angiotensin system (Cuneo et al., 1991c). Fluid retention associated with growth hormone administration has resolved spontaneously when treatment was continued or responded to either a 50% reduction in the daily dose of growth hormone or a change from daily to thrice weekly injections; nevertheless, fluid retention remains a concern in adults receiving growth hormone.

d. BONE. Reduced bone density is characteristic of growth hormone-deficient adults. Long-term studies indicate that growth hormone has positive effects on bone

density in growth hormone-deficient adults (Degerblad et al., 1992; O'Halloran et al., 1993). The results of a study by Degerblad et al. (1992) showed that significant increases in forearm bone density occurred after 12, 18, and 24 months of daily growth hormone injection (0.27 mg/kg/day). The average annual increase in bone density was estimated to be 12%, whereas control patients experienced a 2% average annual decrease in bone density. Similarly, O'Halloran et al. (1993) observed that increased duration of growth hormone treatment was needed to see improved forearm bone density and that spinal trabecular bone was more responsive to growth hormone therapy. Two groups of investigators have reported that growth hormone treatment can increase linear height in growth hormone-deficient adults (Bengtsson et al., 1993; Rudman et al., 1990).

In conclusion, both the type of bone and the treatment duration appear to influence the outcome of growth hormone trials in growth hormone-deficient adults (reviewed by Parfitt, 1991).

The effects of growth hormone administration on bone density in elderly individuals has been studied in trials of shorter duration. In one 6-month trial, growth hormone administration to elderly men resulted in a statistically significant increase in the density of the lumbar vertebrae (Rudman et al., 1990). There was no increase in the radius or proximal femur in this study. In three other 6-month trials, standard daily replacement doses of growth hormone were given to growth hormone-deficient adults. No improvement in bone density was observed despite significant elevations in serum IGF-I levels (Aloia et al., 1976; Binnerts et al., 1992; Cuneo et al., 1991a).

e. HORMONES. Elevations in serum IGF-I and insulin levels occur consistently in adults following growth hormone administration (Bengtsson et al., 1993; Jorgensen et al., 1991; Rudman et al., 1990; Salomon et al., 1989). The increased serum insulin does not result in hypoglycemia nor is abnormal glucose tolerance generally observed. The increase in insulin is thought to be a compensatory effect secondary to a growth hormone-induced state of insulin resistance. Fasting serum glucose levels were slightly elevated in one study (Rudman et al., 1989), but most studies show no abnormalities in glucose metabolism with replacement doses of human growth hormone.

Growth hormone administration alters thyroid hormone metabolism. There is a decrease in serum thyroxine and an increase in thyroid hormone suggestive of an enhanced conversion of thyroxine to triiodothyronine. Alterations in thyroid hormone metabolism could predispose adults to cardiac side effects and should be monitored (Bengtsson et al., 1993).

f. SERUM LIPID PROFILES. Serum cholesterol, triglycerides, LDL, and very low-density lipoproteins are elevated in many growth hormone-deficient adults (Merimee et al., 1972). The absence of growth hormone in these individuals is thought to unmask a genetic predisposition to hyperlipidemia of the familial combined hyperlipoproteinemia type (Merimee and Pulkkinen, 1980). The alterations in serum lipid profiles reported in these individuals are known risk factors for cardiovascular disease and may contribute to the increased cardiovascular mortality noted in growth hormone-deficient adults (Rosen and Bengtsson, 1990).

The usefulness of growth hormone replacement therapy for the reduction of serum cholesterol levels in adults is not established. Statistically significant reductions in serum cholesterol and/or LDL in growth hormone-deficient adults by growth hormone replacement therapy have been reported by some (Binnerts et al., 1992; Salomon et al., 1989), but not all (Degerblad et al., 1992; De Muinck Keizer-Schrama et al., 1992; Merimee and Pulkkinen, 1980; Rudman et al., 1990), investigators. No changes in high-density lipoprotein levels have been observed (Binnerts et al., 1992).

Binnerts et al. (1992) reported that near-maximal reductions in serum cholesterol levels were apparent after the first month of growth hormone treatment. Therefore, variable treatment duration cannot explain response failures. The influence of age, the severity of the hyperlipoproteinemia, and the growth hormone dose are variables that could account for the observed differences in cholesterol response in the subject population and merit additional study.

Older patients receiving growth hormone (0.1 mg/kg/day for 7 days) showed significant elevations in serum triglyceride levels (Friedman et al., 1972, 1974).

2. *Metabolic effects in normal adults.* There is interest in the effects of human growth hormone in normal young adults. The use of growth hormone in this population is purely pharmacological because normal endogenous circulating growth hormone levels are present.

Growth hormone is a well-established anabolic agent in normal young men and women. Nitrogen retention, protein synthesis, and lean body mass are increased and adipose tissue are decreased by growth hormone administration (Crist et al., 1991). These properties of growth hormone have led to experimentation with growth hormone as a supplement to caloric restriction for weight loss (Snyder et al., 1988). The use of growth hormone in obese individuals does not promote increased weight loss, and fluid retention caused by growth hormone may counteract the effects of dieting on body weight (reviewed by Wilson, 1992).

Growth hormone supplementation during physical conditioning training does not enhance exercise-stimulated increases in muscle strength, muscle size, or muscle protein synthesis (Yarasheski et al., 1992; Deyssig et al., 1993). Overnight serum growth hormone levels in individuals treated with growth hormone (0.04 mg/kg/day) were 6 times greater than in the control population. This

dose of growth hormone stimulated increases in IGF-I levels that were 4 times higher than controls. Increases in lean body mass were induced by growth hormone supplementation during physical conditioning but were not the result of increased skeletal muscle (Yarasheski et al., 1992). There is presently no published information concerning the ability of growth hormone to enhance maximum exercise performance or cardiac output in normal young adults.

There are several reports that growth hormone treatment improves the lipid profile in normal young adults and in hypercholesterolemic individuals. In young physically fit men and women (average age, 28 years), total serum cholesterol was significantly reduced from 186 to 166 mg/100 ml with a growth hormone dose of 0.08 mg/kg given three times/week for 3 to 6 weeks (Crist et al., 1991). Both high-density lipoprotein and LDL levels were significantly reduced in these individuals. No effect on serum triglycerides was observed. In type A (coronary prone) hypercholesterolemic individuals (average age, 48 years), growth hormone administration (0.1 mg/kg/day for 7 days) reduced average serum cholesterol from 300 to 241 mg/100 ml (Friedman et al., 1972, 1974).

The results of these studies indicate that growth hormone decreases serum cholesterol in normal and hypercholesterolemic adults. The effect is rapid and persists with continued growth hormone administration; however, cholesterol levels return to pretreatment levels when growth hormone administration is discontinued. The therapeutic benefit of growth hormone may be offset by the associated risk of fluid retention, glucose intolerance, and triglyceride elevations. The patient population that would benefit most from the cholesterol-lowering effects of growth hormone would be the most at-risk population for cardiovascular sequelae secondary to these potential side effects of growth hormone.

3. Treatment of infertility. Therapeutic effects of growth hormone in female infertility have been observed with alternate day (three times/week) administration of 10 to 65 mg/dose by intramuscular injection (reviewed by Cristman and Halme, 1992; Jacobs, 1992). The optimum dose of growth hormone has not been defined. There are some reports that the addition of growth hormone to standard ovulation induction regimens has increased the pregnancy rate in women undergoing treatment for infertility due to polycystic ovary disease (Ibrahim et al., 1991; Owen et al., 1991b). However, not all investigators have found growth hormone to be of therapeutic value in infertility (Levron et al., 1993; Katz et al., 1993). Growth hormone has also been added to in vitro fertilization protocols to stimulate superovulation prior to oocyte collection (Owen et al., 1991a). This results in the development of more and larger follicles and greater yields of mature oocytes (Owen et al., 1991a; Yoshimura et al., 1993).

Abnormal growth hormone secretory responses to arginine or clonidine may identify women in whom growth hormone supplementation would enhance fertility (Menashe et al., 1990; Ovesen et al., 1992). These data imply that in some women infertility may be the result of inadequate growth hormone secretion. Nevertheless, there are few clear-cut differences in circulating growth hormone levels in fertile as compared with infertile women (Katz et al., 1993).

Preliminary investigations indicate that growth hormone may also be of use in the treatment of male infertility (Shoham et al., 1992). Hypogonadal men with pituitary deficiencies in luteinizing hormone and follicle-stimulating hormone production have low serum testosterone levels and are infertile. Sperm counts are either low or absent in these individuals because testosterone is required for spermatogenesis. Testosterone synthesis, spermatogenesis, and fertility are stimulated in most patients by gonadotropin replacement therapy. A subset of patients resistant to gonadotropin therapy have responded to the combination of gonadotropin replacement with growth hormone supplementation (10 mg, intramuscularly, three times/week). The mechanism of action of growth hormone in male infertility, analogous to that in female infertility may be to stimulate IGF-I production and to sensitize the testis to gonadotropin action (Shoham et al., 1992).

4. Anabolic effects of growth hormone in chronic illness. Growth hormone therapy is beneficial in a number of clinical situations when catabolic metabolism occurs (reviewed by Clemmons and Underwood, 1992; Hindmarsh et al., 1991; Lamberts et al., 1992;). Catabolic metabolism is characterized by a negative nitrogen balance and muscle wasting that is not reversed when a high protein diet is administered. Patients suffering from postsurgical stress, sepsis, burns, and cancer experience negative nitrogen balance and often receive total parenteral nutrition with a high nitrogen component in an attempt to offset the muscle wasting. The addition of growth hormone therapy (0.05 to 0.2 mg/kg/day) to total parenteral nutrition has reversed the catabolic state in postsurgical patients (Hammarqvist et al., 1992), burn patients (Wilmore et al., 1974), and patients receiving total parenteral nutrition for gastrointestinal disease (Ziegler et al., 1992) and malnutrition associated with hemodialysis (reviewed by Hakim and Levin, 1993). Growth hormone therapy has reversed weight loss in patients with cancer (Wolf et al., 1992) and chronic pulmonary obstruction (Pape et al., 1991). Preliminary results suggest that combination therapy with IGF-I and growth hormone may be more beneficial than growth hormone alone in the reversal of catabolic states (Kupf et al., 1993).

Individuals who receive chronic treatment with glucocorticoids also exhibit muscle wasting and negative nitrogen balance. Growth hormone reverses the catabolic state associated with chronic corticosteroid therapy (Horber and Haymond, 1990). This is an important

finding because of the number of patients dependent on long-term glucocorticoid treatment for immunosuppression. Other therapeutic actions of growth hormone in patients with chronic illness include the acceleration of wound healing in surgical and burn patients (Herndon et al., 1990; Garrel et al., 1991).

D. Risks Associated with Human Growth Hormone Use

Recombinant growth hormone has proven to be remarkably free of side effects in children. The single concern has been whether children receiving growth hormone have an increased risk of developing leukemia. This issue has been analyzed extensively and is still being closely monitored (Ogilvy-Stuart and Shalet, 1992; Stahnke, 1992). Nevertheless, the current consensus is that growth hormone treatment does not, in itself, increase the incidence of leukemia in children. Rather, children who are currently eligible for growth hormone treatment bear a greater than average risk for leukemia. Children at known increased risk are those who have had cranial irradiation for a previous leukemia or brain tumor and those who are growth hormone deficient following the removal of pituitary tumors. Another factor that may contribute to the risk of leukemia in growth hormone-deficient children is a deficit in immunosurveillance. Although the role of growth hormone in the human immune system is still quite unexplored, growth hormone has been shown to stimulate natural killer cell activity in human lymphocytes (Crist and Kraner, 1990).

Several metabolic side effects of growth hormone treatment not apparent in children (DiMartino-Nardi et al., 1993) are prominent in adults. Fluid retention is a consistent side effect among all adult populations receiving growth hormone therapy. The severity of this side effect can be controlled by dosage reductions, and it resolves spontaneously after growth hormone administration is stopped (Binnerts et al., 1992; Degerblad et al., 1992; Salomon et al., 1989). Elevations in serum glucose are reported in some, but not all, studies (Degerblad et al., 1992; Salomon et al., 1989). Increasing age may be a predisposing factor in the incidence of glucose intolerance.

Growth hormone administration to adults also has implications with regard to cancer incidence. Deaths due to cancer are significantly increased in agromegalics and significantly decreased in growth hormome-deficient adults (reviewed by Ezzat and Melmed, 1991; Bengtsson et al., 1988). It has been hypothesized that long-term elevation of IGF-I levels induced by chronically elevated growth hormone levels could promote cancer growth in acromegalic patients. IGF-I is mitogenic for human breast cancer cells, and production of IGF-I by human bone, kidney, liver, colon, and mesenchymal tumors has been demonstrated (reviewed by Ogilvy-Stuart and Shalet, 1992). In addition, growth hormone itself has been shown to stimulate proliferation of normal and transformed human lymphoid cells in vitro (reviewed by Stahnke, 1992). At the present time, the question remains whether growth hormone given chronically to adults would increase their cancer risk. Data are not available to address this issue because no adults have received long-term growth hormone therapy.

The chronic use of growth hormone by athletes bears all the risks associated with acromegaly because pharmacological levels of growth hormone are usually taken. These include cardiac, renal, and splenic hypertrophy, cardiac myopathy, fluid retention, glucose intolerance, abnormal bone growth, and increased cancer risk.

E. Therapeutic Alternatives to Therapy with Human Growth Hormone

Clear indications for growth hormone therapy exist in children and adults. For long-term usage, the expense of recombinant human growth hormone ($10,000 to 15,000/year/growth hormone-deficient child) and the requirement for daily injections are major disadvantages. Most cases of human growth hormone deficiency result from growth hormone secretory defects rather than a deficit in pituitary hormone production (reviewed in Pintor et al., 1989). The use of growth hormone-releasing agents as alternatives to human growth hormone replacement is being investigated.

Growth hormone administration to adults, particularly the elderly, may be much safer if growth hormone derivatives could be synthesized that retain the desired biological activities and lack the undesirable elevations in extracellular volume and serum glucose. Identification of protein domains responsible for the growth and diabetogenic actions of growth hormone is being investigated by site-directed mutagenesis (Towns et al., 1992).

1. Growth hormone-releasing factor. Only the first 29 amino acids of GRF are required for growth hormone-releasing activity. Based on the smaller molecular size of GRF, it was hypothesized that synthetic GRF analogs less expensive than growth hormone and absorbed without injection could be developed as alternatives to growth hormone replacement (reviewed by Frohman and Jansson, 1986). Continuous intravenous administration or pulsatile (twice daily) subcutaneous injections of GRF in humans stimulated levels of circulating IGF-I and growth. Twice daily subcutaneous injections of GRF were administered to slowly growing children demonstrating inadequate spontaneous growth hormone secretion (Lifshitz et al., 1992). Two-thirds of these patients showed accelerated growth during a 1-year treatment period. Interestingly, some children continued to grow at an accelerated rate after treatment had ceased, suggesting that short-term GRF administration could have long-term beneficial effects on growth rate.

Intranasal administration of native GRF was 100 to 300 times less effective than intravenous administration because of peptide degradation (Ross et al., 1989). In an

effort to increase the efficacy of GRF therapy, various synthetic GRF analogs have been developed with higher receptor affinity and increased resistance to proteolytic degradation (Bongers et al., 1992; Su et al., 1991). Controlled studies comparing the efficacy of growth hormone and GRF analogs in human growth have not been published. In conclusion, GRF and GRF analogs show promise as alternatives to growth hormone in some patients.

2. Clonidine. Clonidine is an orally active stimulant of growth hormone release. Given once a day at bedtime, clonidine simulated the physiological increase in serum growth hormone associated with the onset of slow-wave sleep and exhibited no adverse side effects in adults or children. Several studies indicated that clonidine administration during a 6- to 12-month period stimulated a sustained increase in growth velocity in slowly growing children with growth hormone secretory dysfunction (Pintor et al., 1989; Thorner et al., 1987). In one recent study of short (fifth percentile or less) prepubertal boys, nightly clonidine treatment for 6 months had no effect on growth velocity, although after shifting to growth hormone, all boys showed significant increases in growth velocity (Allen, 1993). The discrepant results suggest differences in responding patient populations or study design. The efficacy of clonidine as a replacement for human growth hormone in children and adults will require additional investigation.

3. GHRP-6 and L-692,429. GHRPs are a class of hexameric peptide that stimulate growth hormone release in children and adults (Momany et al., 1984; Penalva et al., 1993). Presently, GHRP-6 is not an approved human therapy. However, no adverse effects of GHRP-6 have been reported, and it holds promise as a therapeutic agent.

GHRP-6 is thought to stimulate growth hormone secretion by binding to specific receptors in the hypothalamus and pituitary distinct from GRF receptors and by increasing GRF (Bowers et al., 1984, 1991). GHRP-6 and GRF stimulate growth hormone release synergistically (Bowers et al., 1990). The response to GHRP-6 is also significantly enhanced in the presence of the α_1-adrenergic antagonist, prazosin (Penalva et al., 1993). GHRP-6 may also act functionally as a somatostatin antagonist, causing membrane depolarization via blockade of potassium channels (Smith et al., 1993).

L-692,429 is a nonpeptide inducer of growth hormone secretion with a mechanism of action that is indistinguishable from that of GHRP-6 (Smith et al., 1993). L-692,429 may be therapeutically useful as an orally active growth hormone secretagogue.

V. Pathophysiological Consequences of Growth Hormone Excess

Acromegaly is the pathophysiological consequence of growth hormone excess. This disorder is nearly always the result of a growth hormone-secreting pituitary adenoma, although rare growth hormone-releasing hormone-secreting tumors producing acromegaly have been described in the literature. Of all functional pituitary adenomas that hypersecrete hormones, growth hormone-secreting adenomas are the second most common, after prolactin-secreting lactotroph adenomas. Histologically, growth hormone-secreting adenomas are acidophilic and may have either a sparsely or densely granulated appearance; immunostaining may reveal production of other pituitary hormones, particularly prolactin. Growth hormone-secreting adenomas are rarely malignant. The histological appearance of these tumors often correlates poorly with clinical behavior, and, therefore, other parameters, such as biochemical measurement of growth hormone and IGF-I and radiographic appearance, aid in the characterization and management of these tumors (Thapar et al., 1993).

Patients with acromegaly may have clinical manifestations that are either the direct result of growth hormone/IGF-I excess or the consequence of local destruction of normal adjacent pituitary gland and/or other surrounding neurological structures. Recognition of acromegalic changes can often be quite subtle and may not be recognized by patient or family members. Comparison of serial retrospective photographs (over several months to years) may provide clues about the onset and development of acromegaly. Postpubertal effects of growth hormone excess on bone include acral enlargement of the hands and feet, which the patient may note by increasing glove, ring, or shoe size. Enlargement of the skull can produce frontal bossing, and growth of the mandible produces prognathism. When growth hormone-secreting tumors occur prior to pubertal closure of the epiphyseal plates, the result is pituitary gigantism, a rare condition that leads to excessive long bone growth and tall stature in children and adolescents (Daughaday, 1992).

A degenerative arthropathy is also associated with growth hormone excess, which usually affects the knee, but can also occur in the shoulder, hip, ankle, elbow, wrist, and hand. Radiographic appearance of the acromegalic osteoarthropathy includes widened joint spaces, due to excess cartilage growth, along with osteophyte formation, mineralization of ligamentous insertions (enthesopathy), and joint capsule calcifications (Lieberman et al., 1992).

Soft tissue enlargement and organomegaly also occur in conditions of growth hormone excess. In addition to frontal bossing and prognathism, acromegalic facies also include increased nasolabial skin folds and enlargement of the lips and tongue. Enlargement of soft tissues of the upper airway can lead to the development of sleep apnea in persons with acromegaly (Grunstein et al., 1991). Skin tags may be a prominent dermatological feature. Excessive soft tissue growth may compress the median nerve at the wrist, producing carpal tunnel syndrome, a peripheral motor/sensory neuropathy. A proximal myopathy

may occur, producing exercise intolerance and weakness. Cardiomegaly, which can occur independently of associated hypertension, may result in left ventricular hypertrophy and diastolic dysfunction, which are reversible following treatment of growth hormone excess (Lim et al., 1992). Electrocardiographic abnormalities and malignant arrhythmias may also develop in those with acromegaly.

Several recent studies have shown an association between acromegaly and the development of colonic neoplasms. Retrospective analysis has noted an increased incidence of premalignant colonic polyps and colon cancer in persons with acromegaly, particularly in individuals with other identifiable risk factors; these include a family history of colon cancer, age >50 years, male gender, and more than three skin tags present (Molitch, 1992). The mechanism by which growth hormone or IGF-I may modulate this association has not been determined.

Other clinical manifestations of acromegaly are due to the metabolic effects of growth hormone excess. Glucose intolerance, and in some cases frank diabetes mellitus, occurs in as many as half of all patients with acromegaly. The etiology of this diabetogenic effect is not clear but may be due to growth hormone-induced insulin resistance. Hypertension occurs in approximately one-third of all cases of acromegaly and appears to be associated with an expanded extracellular fluid volume. This may, in part, be due to the actions of growth hormone at the renal proximal tubule, which results in sodium retention, an effect that appears to be independent of the renin/aldosterone axis (Molitch, 1992).

Some of the clinical manifestations are due to local invasive destruction of the pituitary gland, leading to endocrine abnormalities that include secondary hypothyroidism, adrenal insufficiency, and hypogonadism. Visual field defects may be produced by suprasellar extension of the growth hormone-secreting adenoma, as it impinges on the adjacent optic chiasm. Frontal headaches, which may be partly due to tumor expansion, are a common symptom in acromegaly. Decreased libido and impotence in men, and menstrual cycle disturbances in women can also result from mild hyperprolactinemia, which may result from disrupted inhibitory efferent neuronal pathways to the pituitary. However, galactorrhea, which is also common in women with acromegaly, is the result of the lactogenic actions of growth hormone.

Diagnosis and management of acromegaly is beyond the scope of this review, but in addition to neurosurgical resection and pituitary irradiation, two main therapeutic options exist for primary and adjuvant pharmacological treatment of growth hormone-secreting pituitary adenomas. Neither drug is tumoricidal, but both have shown efficacy in decreasing tumor size and growth hormone/IGF-I levels to varying degrees.

Bromocriptine is an ergot alkaloid dopamine receptor agonist that inhibits growth hormone secretion from somatomammotropic cells in the pituitary. The molecular mechanism of its pharmacological action is not known. Although bromocriptine has traditionally been regarded as more effective therapy in the treatment of prolactinoma, similar effects can be achieved at somewhat higher doses of bromocriptine (up to 20 mg/day) (Melmed, 1990). One large study found that 54% of patients treated with bromocriptine achieved growth hormone suppression to <10 μg/liter, but less than 20% had tumor shrinkage (Barkan, 1989). However, 70% of patients noted subjective improvement in several symptoms of acromegaly.

The other major pharmacological therapy used extensively to treat acromegaly is octreotide, a modified 10-amino acid somatostatin analog that suppresses growth hormone release, in addition to several other gastrointestinal/pancreatic polypeptides. Although it can be given orally, octreotide's low bioavailability by this route of administration makes it prohibitively expensive; therefore, it is usually administered by subcutaneous injections every 6 to 8 hours or by continuous subcutaneous infusion. Octreotide can suppress growth hormone levels to normal in up to 30 to 40% of patients and can shrink tumor size in both micro- (<10 mm) and macroadenomas (>10 mm) (Frohman, 1991). It is not possible to predict which drugs will prove efficacious in any individual case.

VI. Summary

The study of human growth hormone is a little more than 100 years old. Growth hormone, first identified for its dramatic effect on longitudinal growth, is now known to exert generalized effects on protein, lipid, and carbohydrate metabolism. Additional roles for growth hormone in human physiology are likely to be discovered in the areas of sleep research and reproduction. Furthermore, there is some indication that growth hormone also may be involved in the regulation of immune function, mental well-being, and the aging process. Recombinant DNA technology has provided an abundant and safe, albeit expensive, supply of human growth hormone for human use, but the pharmacological properties of growth hormone are poor. Most growth hormone-deficient individuals exhibit a secretory defect rather than a primary defect in growth hormone production, however, and advances in our understanding of the neuroendocrine regulation of growth hormone secretion have established the basis for the use of drugs to stimulate release of endogenously synthesized growth hormone. This promises to be an important area for future drug development.

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