Biosynthesis of Growth Hormone and Insulin-Like Growth Factor-I and the Regulation of their Secretion

A. Skottner*

AstraZeneca R&D, Södertälje, Sweden

Abstract: This report reviews the current knowledge on the biosynthesis, regulation and secretion of Growth Hormone (GH) and Insulin-like Growth factor-I (IGF-I). Variants of human GH, molecular size variants as well as charge variants, and the GH binding protein will be discussed, and the regulation of expression of the *hGH* gene is reviewed. IGF-I expression, regulation of the *igf-I* gene and secretion is discussed and a separate section on the influence of the six IGF binding proteins is included.

Keywords: hGH, IGF-I, biosynthesis, secretion, regulation, binding proteins.

INTRODUCTION

Both GH and IGF-I play important roles in growth and metabolism throughout life. GH is essential for postnatal growth in all species, but does also exert important peripheral effects on energy metabolism, body composition, bone metabolism, immune system, muscle function as well as on the central nervous system (CNS), affecting e.g. appetite, cognition and sleep. IGF-I is a mitogen and stimulator of cell proliferation, and has important functions in tissue repair and regenerative processes. It also mediates some of the anabolic and growth activities of GH.

Much information on how and where these hormones are produced in the body and the regulation of their expression, as well as how and where GH and IGF-I exert their biological action has been gathered. Specific receptors in certain tissues and cells have been identified, and soluble binding proteins carrying the peptides in plasma, prolonging the half-lives and making the active peptides available or inhibiting their actions have been established. Results from *in vitro* studies in different cell types, studies in experimental animals, the use of transgenic techniques and human clinical trials, have revealed the importance of GH and IGF-I in many physiological functions, not only growth and metabolism, but also on development, regeneration and the immune system. It is also evident that both GH and IGF-I have important actions in the neurological system, both in the CNS and peripheral nervous system, impacting development, cognition and mood, and exerting neuro-protective activity. This Chapter gives a brief overview of the current knowledge on the expression of GH and IGF-I and the regulation of their secretion.

*Address correspondence to this author at the AstraZeneca R&D, Regulatory Affairs, B213, Forskargatan 20, SE-15185 Södertälje, Sweden; Tel: +46855324144; Fax: +468555328909;
E-mail: anna.skottner@astrazeneca.com

GROWTH HORMONE

The first signs of a pituitary factor involved in growth were already identified a century ago by Crowe *et al.* [1], who described the induction of growth arrest after hypophysectomy. Later, Evans [2, 3] reported that administration of bovine pituitary extracts restored growth in female rats, and that a 'growth hormone' would be contained in the pituitary. Human GH (hGH) was first isolated by Li and coworkers [4] and the structure of the peptide was elucidated [5]. Human GH is a single chain peptide, comprising 191 amino acid residues, with two disulphide bonds and a molecular weight of 22,000 daltons (22K). The human GH gene is found on the long arm of chromosome 17. Two different loci coding for hGH were identified in the gene cluster, *hGH-N* and *hGH-V* [6]. The corresponding peptides resulting from these genes are highly homologous but differ at 13 amino acid residues throughout the peptide chain, and are expressed differently. *Human GH-N* is expressed primarily in the pituitary, but also found in several other tissues, although to a smaller extent, and *hGH-V* is expressed in the placenta during pregnancy [7]. In addition to the main form of hGH, several different variants of hGH has been identified, differing in charge and size [8, 9].

Molecular Weight and Charge Variants

20K is a hGH isoform produced in the pituitary by alternate splicing of the GH mRNA having a molecular weight of 20,000 daltons (20K) and lacking 15 amino acids in position 32 to 46. The 20K form of hGH comprises between 6 and 10 % of the hGH present in the pituitary and was first described by Lewis *et al.* [10]. The 20K variant has been shown to exert similar effects on growth in experimental animals [11], and metabolism in humans as the normal 22K hGH [12], but has shown poor lactogenic activity. Several high molecular weight (big, big-big), and oligomeric variants of hGH and 20K have been isolated, among them dimeric forms, both covalently and

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non-covalently bound variants [13]. High molecular weight variants of GH have been isolated from pituitaries and from cultures with pituitary cells, but also from plasma, thus, indicating a wide distribution of variant GH's secreted. The oligomeric variants of GH demonstrate a variable, but mainly decreased, biological activity compared to the monomeric forms [14, 15].

A number of charge isoforms of growth hormone have furthermore been isolated, mainly from pituitary extracts. These isoforms include deamidated and amino-acetylated variants [16]. Deamidation has been shown to occur at amino acid residues 137 and 152 [17]. Deamidation of the hGH molecule was shown to make it more prone to proteolytic cleavage, and two-chain variants of the hormone have been identified [18]. The biological action of the charge isomers were shown to exert similar growth-promoting and diabetogenic activity as native pituitary hGH in rodent models [19]. A glycosylated variant of hGH has also been isolated [20], but the bioactivity of this variant is still not fully elucidated.

Placental GH, hGH-V

Placental GH, hGH-V, is a variant GH which is produced in the placenta during pregnancy. It differs from hGH-N in 13 amino acid residues, and has a higher isoelectric point. The hormone is composed of two variants, one of which is glycosylated and has a slightly higher molecular weight. In contrast to pituitary GH, it is secreted in a non-pulsatile manner, and secretion rises continuously during the second and third trimester of pregnancy, simultaneously inhibiting pituitary GH secretion. The concentration of hGH-V falls immediately at the onset of labor, as well as at cesarean section. Placental GH is only detected in maternal blood, not in fetal circulation. Recent studies have further demonstrated the availability of placental GH in amniotic fluid [21]. The factors regulating the production of placental GH are merely unknown, but increased maternal glucose decrease secretion of placental GH [22]. The main function of hGH-V may be on placental development and nutrient availability to the fetus [9, 23, 24].

GROWTH HORMONE BIOSYNTHESIS

The genes encoding human GH is found on chromosome 17, and is part of a cluster of five genes, including the genes for the two GHs, *hGH-N, hGH-V*, and Chorionic somatomammotropin (*CS* or Placental Lactogen). The genes show a different pattern of tissue-specific expression in the anterior pituitary somatotrophs (*hGH-N*) and in the placental syncytiotrophoblasts (*hGH-V* and *CS*) [25]. Further data have shown that GH is also expressed in other peripheral tissues, probably exerting auto-or paracrine functions in the local environment [26].

The expression of the *hGH-N* gene is controlled by transcription factors, among which *Pit-1* plays a central role [27]. Inactivation or lack of functional *Pit-1* expression in both mice and man inhibits the differentiation and proliferation of the pituitary cells [28]. Several studies have revealed that *Pit-1* complexes at DNaseI hypersensitive sites (HS), specifically at the HS-I site, establishing a acetylated histone domain which potentiates *hGH-N* transcription [29-31]. HS-I is located within the locus control region (LCR) 5' to the *hGH-N* gene, and was recently shown to be crucial for establishing a domain of non-coding polymerase II (PolII) transcription, which is linked with chromatin organization of the *hGH-N* locus, thus, constituting a robust pathway for gene activation [32].

However, expression and binding of *Pit-1* is not enough for GH transcription, as other transcription factors are also required and cooperate with *Pit-1*. Such factors are the Sp1 protein, which also binds the GH promoter, assisting *Pit-1* to bind to its site and *Zn-15* which seem to function synergistically with *Pit-1* in order to activate the GH promoter [33]. Other transcription factors involved in the regulation of GH expression are cAMP response element binding protein (CREB) [34], the activator protein (AP2) [35] and possibly the upstream stimulatory factor (USF) [36]. Studies to determine interactions between specific transcription factors and GH mRNA abundance revealed a significant correlation for *Pit-1*, *Prop-1* and *Zn-16*, all of which were significantly decreased (or not detected) in hypopituitary (Ames or Snell dwarf) mice strains, but increased to approximately 200% in mice overproducing GH [37]. *Prop-1* has been demonstrated to activate *Pit-1* expression in the pituitary, but was also suggested to have other (functional) roles since low levels of *Prop-1* mRNA were detected in normal mouse pituitaries. The exact function of *Zn-16* is not fully elucidated, but the significant correlation with eg. GH mRNA may predict a transcription factor which synergize with somatotrophic *Pit-1*. Factors that are negatively involved in the regulation of GH expression is the zinc finger Ikaros (*Ik1*) which deacetylates histone 3 residues in the GH promoter and limits the access of *Pit-1*, thus limiting GH expression [38], and Thyroid transcription factor1 (*TTF-1*), which binds to the promoter region of the GH gene and inhibit transcription [39]. DNA methylation is another (negative) regulator of GH gene transcription [40, 41].

Although *Pit-1* is mainly expressed in the pituitary cells, it has also been demonstrated in extrapituitary tissues, including lymphoid tissue and haemapoietic cells but also mammary gland cells (normal and breast cancer cells). Recent data indicate that expression of *Pit-1* in the mammary gland regulates local GH expression and may be involved in mammary development and possibly breast disorders [42].

GH is expressed in the brain, in areas different from the pituitary, such as the hippocampus, cortex, caudate nucleus and retinal areas [43]. Expression has been shown to increase following injury [44] and also in response to learning [45].

REGULATION OF GROWTH HORMONE SECRETION

Growth hormone has for long been considered a classical hormone, exerting endocrine functions. However, it is now accepted, that GH in addition to its expression in the anterior pituitary, is also produced peripherally and exerts both auto- and paracrine actions [26]. Variants of growth hormone, such as the placental GH (hGH-V), are produced locally in

the syncytiotrophoblasts, and exert its effects both in paracrine and endocrine manners.

It is well established that pituitary growth hormone is secreted in a pulsatile manner from the pituitary. The secretory pattern of GH was initially elucidated in rats, demonstrating a distinct difference in GH secretion between males, with low trough levels and high amplitude peaks, and females, showing higher basal levels (troughs) and less pronounced peaks [46]. The pulsatility of GH in male rats is very consistent, with peaks appearing approximately every 3rd hour, whereas the female pattern is less regular. In addition, the difference in GH secretion in males and females, led to the physiological explanation of the corresponding sexual dimorphism resulting from the difference in GH secretory patterns, demonstrating significant differences in linear growth and the hepatic steroid metabolism [47, 48]. The male pulsatile GH pattern, was shown the most efficient for longitudinal growth [49]. In humans, the diurnal secretory pattern of hGH is fully developed after puberty, demonstrating a major peak at late night/early morning which is associated with the REM-sleep, and a number of peaks during the light hours of the day, generally varying between 3 and 6 peaks per day, but with quite large individual difference [50, 51]. Growth hormone secretion is mainly regulated by the concerted action of the hypothalamic factors, Growth hormone releasing hormone (GHRH) [52] and somatostatin (somatotropin releasing inhibiting factor; SRIF) [53] both produced in the hypothalamus and reaching the pituitary *via* the pituitary-portal system [54-56]. Further studies have revealed that SRIF regulates the magnitude of the troughs of GH as well as the amplitude of the peaks, whereas GHRH function as the main regulator of the pulsatile pattern [57, 58].

It is well known that growth hormone can regulate its own secretion. Several studies in animals and humans have shown that GH inhibits its own secretion. This has been demonstrated in humans and rodents during exogenous administration of GH or 20K hGH [59-61], and during pregnancy, when maternal circulating levels of placental GH increases, and the pulsatile secretion of pituitary GH disappeared [62], The mechanism behind the inhibition may be two-fold, depending on whether it is a short term inhibitory loop or if the inhibition is longer term. The short term inhibition is most likely due to GH-induced stimulation of SRIF release and inhibition of GHRH secretion [63, 64]. However, in the long term feedback situation, the inhibition of GH secretion is most likely due to feedback inhibition by IGF-I. Infusion of IGF-I induced a decrease in total plasma levels of GH by 50-80% of initial levels in both males and females, and more or less abolished the pulsatile secretion [57]. Interestingly, IGF-I infusion suppressed GHRH induced GH release in males, but not in females, suggesting a sexually dimorphic effect. Thus, the results suggest that increased plasma levels of IGF-I suppress GH secretion at the hypothalamic level in females, whereas in males suppression may be either at the pituitary or hypothalamic levels. Others have proposed that in the hypothalamus, mainly locally produced IGF-I is responsible for the feedback inhibitory actions on GH release, eg by enhancing SRIF release. Available data further demonstrated the lack of effects on the expression of hypothalamic GHRH and SRIF in rodents depleted of IGF-I produced in the liver [65]. *In vitro* studies, using pituitary cells, revealed a significant inhibitory action on GH secretion of IGF-I in all species tested. Based on current knowledge, it seems as if most of the IGF-I feedback regulation on GH secretion is maintained *via* the pituitary.

The gastric peptide ghrelin has been identified as a stimulator of GH release. Ghrelin mRNA was detected primarily in the stomach, but has also been demonstrated in other cells, including the hypothalamus [66], and stimulates the secretion of GH, although several other functions especially affecting food intake and metabolism have been attributed to this factor. Recent data suggests that the presence of hypothalamic GHRH is needed for the full action as demonstrated by the use of synthetic ghrelin analogues [57]. Others have proposed that, in spite of only limited endogenous regulatory functional effects on GH secretion, ghrelin may act as link between metabolic status and growth [67].

Other studies have demonstrated the importance of dopamine, cathecholamines and cholinergic factors in the regulation of pituitary GH secretion, which regulate GH *via* GHRH and SRIF, respectively. Furthermore, glucocorticoids inhibit GH secretion, probably *via* increased expression of SRIF [68]. Sex hormones affect the production of GH, acting both at hypothalamic, pituitary and peripheral levels. They are important factors for the increased GH secretion observed during puberty (see below) but also later in life. Both estrogen and testosterone increase GH secretion in humans by amplifying secretory burst mass and reduce the orderliness of the GH secretion [69]. Estrogen affects GH secretion mainly by interacting with the estrogen receptor (ER) ERα, expressed in the GHRH neurons and in GH-secreting pituitary cells. It has been proposed that the stimulatory effects of estrogen on GH secretion is mediated *via* the release of GHRH and/or by enhancing the sensitivity to ghrelin released from the hypothalamus [70].

Further endogenous and exogenous factors that influence the secretion of GH have been identified. Examples of such factors are arginine, insulin (hypoglycemia), clonidine, levodopa and pyridostigmine, some of which have been used clinically to identify potential pituitary insufficiency. Recent studies have revealed that the GH response to all of the above factors is inhibited by GHRH receptor antagonists, thus, indicating that the mechanism by which GH is secreted in response to these stimuli, is *via* GHRH [71].

Pituitary secretion of GH is significantly increased in situations of dietary restriction and fasting. Ho *et al.* [72] reported that a 5-day fast in normal men resulted in significantly increased pulse frequency as well as pulse amplitude. The mechanism behind the increased secretion may be explained by the decreased expression and secretion of IGF-I in response to the fasting, thus, limiting the feedback inhibitory effect on pituitary GH secretion (see above). In addition, leptin, an anorexigenic peptide expressed in adipocytes as well as in the pituitary and other

areas of the brain, has been identified as an important modulator of GH secretion. Leptin exerts differential effects on GH secretion, depending on whether the impact is short-term or long-term [73]. Short term effects of leptin increase GH secretion, mainly by increase in GHRH and decrease in SRIF expression, whereas long-term effects significantly decrease GH secretion, probably due to a decreased response to GHRH. In obese subjects, GH secretion is reduced both in animals and humans, and GH responsiveness is decreased. Interestingly, the pulsatile secretion was decreased in obese rats [74], whereas in humans total daily GH secretion was decreased, but with intact pulsatility [75]. Leptin plasma concentrations are constantly increased, which may be part of the explanation to the decreased GH secretion in obesity [76].

GH synthesis and secretion exhibit major changes with age. The pulsatile secretion is markedly increased in early infanthood, possibly due to low circulating IGF-I levels, as GH sensitivity is low immediately after birth. A major increase in the total secretion of human GH in adolescents is observed at the growth spurt during puberty, which is reflected both in increased peak number and peak amplitude [69]. Pubertal increases in GH are largely associated with changes in the levels of sex hormones. Recent data has revealed that estrogen affects the increase in GH secretion mainly by increasing the irregularity in pulsatility and lowering total and free IGF-I, whereas testosterone rather increases basal GH secretion and IGF-I concentrations, thus, relieving the negative feedback (by IGF-I) on the GH secretion [69]. Correlation analysis showed that (IGF-I and) GH secretory pulse mass directly varied with serum testosterone concentrations [77]. The total amount of hGH secreted daily differs between males and females, being approximately twice as high in females as in males.

GH secretion declines with age in all mammals studied, starting already at early adulthood [78, 79]. The mechanism by which GH is decreased is primarily due to reduction in hypothalamic GHRH secretion, but depletion in sex steroids may also be part of the puzzle.

Several studies, especially in rodents, have recently shown that decreased GH secretion and concomitant reduction in circulating IGF-I is associated with increased longevity. Possible explanations include metabolic changes, e.g. increased insulin sensitivity, with subsequent modulation of glucose and fat metabolism, and decreased susceptibility to stress and carcinogenesis [80]. The age related changes of the GH/IGF-I axis will be discussed in further detail in Chapter 11 of this volume.

GROWTH HORMONE BINDING PROTEINS

The biological effects of growth hormone are mediated *via* the binding of GH to a specific membrane receptor. The GH receptor (GHR) is single-chain, trans-membrane Type I cytokine receptor, expressed in most tissues. It is expressed as a monomer, but for activation by GH, two receptors form a dimer complexing with one GH molecule [81]. Intracellular signaling is mediated *via* the JAK2/Stat5a and Stat5b pathway, but increasing evidence has shown that activation *via* the Src tyrosine kinase family and subsequent activation of extracellular regulated kinases may be an alternate pathway [82]. Nuclear accumulation of GHRs have been reported, especially in different types of cancers.

In humans, slightly less than 50% of GH is bound to high mw carrier proteins in the circulation. To date, two GH binding proteins have been identified, one of which has high affinity and comprises the extracellular domain of the GH receptor and another with low affinity, which has been identified as a α-2 macroglobulin, which may be of less importance [83]. The high-affinity GHBP is mainly produced by proteolytic cleavage of the receptor through ectodomain shedding, although in some species (rats, mice) alternative splicing have been shown. High affinity GHBPs have been demonstrated in many species. However, the function of GHBP is not fully elucidated, but it is likely to be involved in modulating the availability and activity of circulating GH. Results reveal that GHBP blocks binding of GH to the GHR, and thus, inhibit the subsequent biological activity of GH. On the other hand, GHBP significantly prolong the half life of GH in plasma, and it has been shown that GHBP may even improve the activity of GH in some cases [84]. A comprehensive summary of the current knowledge of GH receptors and GHBP is found in Chapter 2 of this volume.

In the brain, GH receptors/GHBP have been demonstrated in mammals in several areas, such as the hypothalamus, hippocampus and spinal cord, and in neuronal as well as non-neuronal cells [44, 85]. It is interesting to note that GHR/GHBPs are to a great extent located in areas involved in neurogenesis [85]. A higher density of GHR/GHBP is generally observed during fetal development and early postnatal life. In man, the highest density of GH binding has been found in the choroid plexus [86, 87], suggesting that GH is transported across the blood-brain-barrier [88]. In humans, the concentration of GH in the CSF has been shown to increase after exogenously administered GH in patients with GH deficiency and high levels of GH have been measured in patients with acromegaly [89].

INSULIN-LIKE GROWTH FACTOR-I (IGF-I)

Initially, IGF-I was identified as the 'sulphation factor', as serum from normal individuals could stimulate *in vitro* incorporation of radioactive sulphate into embryonic chick or normal pig cartilage, but serum from hypophysectomized or growth hormone deficient individuals lacked this effect. No effect was observed when GH was added to the tissue *in vitro*, but serum from GH treated individuals was effective [90]. The factor was further identified as Somatomedin A [91] since it was 'mediating the effects of somatotropin'. Another name was Non-suppressible insulin-like activity (NSILA), as the factor could induce insulin-like activities but the effect was not inhibited by the addition of antibodies against insulin [92]. The final peptide sequence of human IGF-I was elucidated by Rinderknecht & Humbel [93], who simultaneously published the final sequence for IGF-II, a peptide homologue to IGF-I, with slightly different properties. IGF-II, which seems to play an important role in fetal and cancer development, regulating proliferation,

apoptosis and energy production, is not included in this review. IGF-I has a molecular weight of 7.5kD, comprises 70 amino acids with three disulphide bridges. It is a structural homologue to pro-insulin, consisting of two domains connected *via* a C-peptide and an extended E-domain. There is a high homology in the IGF-I amino acid sequences between human and non-human species. Human, porcine, bovine and ovine IGF-Is have identical amino acid sequences, whereas rat and mouse IGF-Is exhibit 96% and 94% sequence homology, respectively [94]. IGF-I from further species have been sequenced and exhibit a high level of similarity with the human hormone, e.g. kangaroo (91%), chicken (89%), salmon (80%) and barramundi (77%) sequence homology. Also their affinity to IGF-I receptors and biological activity seem highly conserved [95].

Already from the early studies it was clear that liver was a major site for IGF production, but later investigations have confirmed the expression and secretion of IGF-I from many different sites and cell types, including the CNS [96, 97]. The expression of IGF-I is mainly modulated by GH, but also other factors are involved in the stimulation of expression and regulation of secretion of the peptide. IGF-I exerts its biological actions in auto-and paracrine manners but also in an endocrine fashion. It does not exhibit any diurnal variation.

IGF-I BIOSYNTHESIS

The gene for IGF-I, *igf-1*, is located on chromosome 12 and consists of 6 exons and 5 introns [98, 99]. The major expression of the polypeptide is found in the liver in most species, but IGF-I is also expressed in almost all cells in the body. Transcription is initiated *via* two promoters, the major promoter (P1) which is active in all tissues and regulates IGF-I mRNAs containing exon 1, and the second promoter (P2) which regulates the transcription of IGF-I mRNA containing exon 2, and is primarily expressed in the liver [100, 101]. Recent data reveal that the exon 1 promoter contains four transcription initiation sites, and the exon 2 promoter has two sites. The two promoters are both found in mammals, whereas in non-mammalian vertebrates only a single promoter is identified in conjunction with exon 1 [102]. Both promoters are activated by GH, and it has been demonstrated that GH induces an alteration in the chromatin structure in the second intron with a single HS site (HS7) appearing prior to IGF-I gene transcription [103]. Further studies revealed two binding sites, with high and low affinity, for Signal transducer and activator of transcription (Stat) 5b in the HS7 region in the mammalian IGF-I gene, and it has been shown that GH induces binding of Stat5b to this region immediately before initiation of IGF-I gene transcription from both promoters [104]. Further results supported the possibility of several Stat5b binding sites, and suggested that apparently redundant response elements may be able to amplify the GH activity for specific genes [105, 106]. Stat5b has also been identified as an important initiator of IGF-I gene transcription in humans, since patients with short stature have been shown to have point mutations in the Stat5b gene [107, 108].

It has recently been demonstrated that the *igf-1* gene can generate several transcripts due to multiple transcription initiation sites, alternate splicing especially at the C-terminal, and different polyadenylation sites. All IGF-I mRNA comprises exons 3 and 4, but splice variants containing exon 4 spliced to exon 6 (designated IGF-IEa) or to exons 5 and 6 (designated IGF-IEb in rodents, but IGF-IEc in humans), respectively, have been observed. It has been proposed that the IGF-IEb mRNA is increased after skeletal muscle injury, with a possible subsequent role in muscle remodeling, and named mechano-growth factor (MGF) [109]. No corresponding peptide has yet been identified *in vivo*, and further work is needed to elucidate the possible role and bioactivity of the different splice variants.

IGF-I is expressed in the CNS [87, 110] as well as in the peripheral nervous system [111]. Within the CNS, IGF-I expression is observed in several regions, being highest during development, but post-natally the major areas in which IGF-I is expressed are the olfactory bulb, hippocampus, and the Purkinje cells in the cerebellum [112]. In adult mammals, expression is generally lower, and further restricted predominantly to the brain stem and cerebellum [110]. Peripherally, IGF-I has been detected in the ventral horn, sympathetic and dorsal root ganglia, axons and Schwann cells of the sciatic nerve in adult rats [111, 113, 114].

In both fetal and adult brain tissue, a truncated form of IGF-I lacking the 3 N-terminal amino acids, has been demonstrated, most likely being a result of posttranslational modifications [115]. Truncated IGF-I (des-N-(1-3)-IGF-I) has further been found in peripheral tissues, and in colostrum. The truncated variant binds only weakly to the IGFBPs, thus, making the peptide readily available for biological action at the receptor site. The amino-terminal tri-peptide, consisting of amino acids Gly-Pro-Glu (GPE), has been identified in human brain as well as in peripheral tissues, and is formed after cleavage by acid proteases. The tri-peptide does not interact with IGF-I receptors or IGFBPs but seem to exert neuroprotective activities *via* the NMDA receptors [116]. GPE may activate NMDA receptors by binding to both glutamate and glycine binding sites, but the precise mechanism of action is not yet fully elucidated. However, results indicate that GPE has comparable antagonistic NMDA effects to the well-known NMDA antagonist AP-5 and that MK801, another NMDA antagonist, blocks the effects of GPE on glial cell proliferation.

IGF-I expression is increased in response to injury. Elevated immunoreactivity has been demonstrated in several tissues, such as peripheral nerves, skeletal muscle and endothelial cells in response to injury in rats [117-119]. A marked increase in IGF-I expression has also been shown in brain after hypoxic-ischemic insults [120], suggesting that locally produced IGF-I acting in an autocrine/paracrine manner is important for local regenerative processes.

REGULATION OF IGF-I SECRETION

The liver is the main production site for plasma IGF-I, but most cells in the body have the possibility of expressing and producing IGF-I [96, 97]. The expression of IGF-I is

regulated by hormones, of which GH plays a major role, oncogenes and other growth factors. Another major factor influencing plasma levels of IGF-I is nutrition.

Growth hormone induces transcription of the IGF-I gene potently and rapidly, especially in the liver. The major mechanism, by which GH induces IGF-I production, appears to be by binding to the GH receptor, activation of the JAK2 pathway and phosphorylation of Stat5b [121]. Although GH is a major regulator of IGF-I gene expression, peripheral synthesis of the peptide is also regulated by other hormones and factors. In osteoblasts, the main regulator of IGF-I expression is PTH, which initiates IGF-I synthesis *via* cAMP [122, 123]. Estrogens are known to increase the expression of IGF-I in different brain regions as well as in peripheral cells. A recent study revealed IGF-I production was regulated *via* the E2α receptor in mouse fallopian tubes [124]. Furthermore, Venken *et al.* [125] showed that estrogen can upregulate hepatic IGF-I synthesis independently of GH, and thereby stimulating pubertal skeletal growth. Conflicting results regarding the effects of glucocorticoids on IGF-I expression are available, and both increased and decreased IGF-I synthesis has been reported. Thyroid hormones, such as T_3 and TSH, enhance the production of IGF-I, and significant decreases of IGF-I expression in different brain regions have been demonstrated after experimental induction of hypothyroidism in fetal mice [126]. PDGF and FGF both increase expression and synthesis of IGF-I.

Nutritional status affects IGF-I plasma levels significantly, and energy depletion or protein starvation lower circulating IGF-I levels. The changes in IGF-I secretion during starvation is independent of changes in pituitary GH secretion, but is likely due to decreased expression and signaling from hepatic GH receptors. However, changes in the pharmacokinetics of IGF-I and IGFBPs have also been observed [127].

Plasma levels of IGF-I are low at birth, slowly rises during childhood, peak just prior to puberty reaching adult levels, and further decreases with increasing age [128]. Postnatally, the age dependent changes in both plasma and tissue IGF-I concentrations are mainly dependent on the age-related changes in GH secretion and GH sensitivity [129], but age-related changes in other hormones, e.g. sex steroids, also impact the synthesis. Fetal plasma levels of IGF-I are generally quite low but raise during the last part of the third trimester. Fetal concentrations of IGF-I are independent of pituitary GH secretion, but associated with placental GH production [130]. Further details of the age related changes of the GH/IGF-I axis will be discussed in Chapter 11 of this volume.

IGF-I BINDING PROTEINS

The majority of IGF-I in plasma is bound to larger carrier proteins. Six binding proteins with high affinity for IGF-I (IGFBP-1 to -6) have been identified and documented in mammals. The binding proteins are modulators of IGF-I activity, acting both as inhibitors and potentiators, as well as a reservoir of IGF-I in plasma. The IGFBPs are subject to proteolysis by different proteases, which reduce the affinity for IGF-I and modulate the availability of the peptide at the receptor level [131]. In addition, they do exert biological actions on their own [132]. IGFBPs are expressed at different levels in many tissues, including the brain, the only exception being IGFBP-1, which does not exhibit CNS expression.

IGFBP-1 and IGFBP-2 both contain an Arg-Gly-Asp (RGD) integrin binding site [133, 134], which likely is important both for enhancing the bioactivity of IGF-I at the cellular level and actions independent of IGF-I [135]. IGFBP-1 is expressed, in addition to the liver, also in the kidney as well as in other tissues such as the endometrium. It circulates in plasma, is mainly regulated by insulin, and either enhance or inhibit IGF-I actions, depending on its state of phosphorylation [132]. IGFBP-2 is expressed in high amount in several tissues and is the most prevalent IGFBP in the nervous system (CNS and peripherally), with the highest expression observed in embryonic tissues and during fetal development in areas of remodelling [136, 137]. In spite of decreased secretion after birth, IGFBP-2 is present in both plasma and CSF throughout life. The main function seems to be inhibition of the IGF-I action [138], although in the brain, IGFBP-2 may act as a facilitator for IGF-I activity [139, 140].

IGFBP-3 is produced in the liver, predominantly expressed in non-parenchymal cells [141], and function as the main carrier of IGF-I in the circulation, prolonging the half-life of the peptide. It is associated with a large glycoprotein, the acid-labile subunit (ALS), expressed in hepatocytes [141], and IGF-I to form a ternary complex [142], and function as a reservoir of IGF-I. However, IGFBP-3 is not confined to plasma, its mRNA has been identified in several tissues apart from the liver, and it has been shown to exert effects locally at a cellular level modulating IGF-I activity, but also exerting IGF-I independent actions [132]. In the CNS, IGBP-3 is expressed only at low levels, mainly in non-neuronal but possibly also in neuronal cells, as shown *in vitro* after GH treatment [143, 144].

IGFBP-4 is expressed in the liver, from which plasma IGFBP-4 is derived, but expression has been shown in several tissues including osteoblasts, fibroblasts, cells in reproductive organs and embryonic cells [145]. In the CNS, only very low levels of IGFBP-4 have been shown, in different brain cells during embryonic development, and postnally mainly in the hippocampus and olfactory bulb [146]. Results from *in vitro* and *in vivo* studies demonstrate mainly an inhibitory action of IGFBP-4 on IGF-I activity [145].

IGFBP-5 is abundant in plasma, and the second most important carrier of circulating IGF-I. Similar to IGFBP-3, IGFBP-5 forms a ternary complex with ALS. IGFBP-5 is to a great extent expressed in the same tissues as IGFBP-3. In the CNS, IGFBP-5 is highly expressed during embryonic development of the brain as shown in rats [147, 148], but is also shown to be an important player in bone development [149] and in tumor biology [150]. A potentiating effect on

IGF-I activity has been demonstrated by IGFBP-5, and interestingly, IGF-I itself increases the expression of IGFBP-5 [132, 147, 151]. Both IGFBP-3 and IGFBP-5 have further been shown to bind to nuclear sites and exert IGF-I independent actions [132, 152, 153].

IGFBP-6 is mainly expressed peripherally, and is expressed within the CNS at very low levels. The IGFBP-6 has a preferential binding for the IGF-I homologue IGF-II, and seems mainly to function as inhibitor of IGF-II action in different IGF-II dependent cancers [154].

FUTURE STRATEGIES

Most of the data available on the expression and actions of GH is based on its secretion from the anterior pituitary and endocrine function. Similarly, data on the expression, secretion and regulation of IGF-I is based mainly on results from peripheral organs, the liver being the major organ for the production of plasma IGF-I. However, during the last decades an increasing amount of data on the biosynthesis and function of GH and IGF-I in the CNS have emerged. It has been demonstrated that GH is expressed locally in certain areas of the brain, apart from the pituitary, and exert specific actions either directly or *via* the induction of locally produced IGF-I. IGF-I is expressed in several CNS areas, acting mainly in an autocrine and/or paracrine manner. Both hormones have furthermore been shown to pass the blood-brain-barrier, therefore, also peripheral changes in GH and IGF-I concentrations may significantly influence the brain. The function of GH and IGF-I within the CNS include important effects on energy metabolism, neurogenesis, neuroprotection, regeneration, cognition and mood. However, detailed studies on the specific regulation of expression and synthesis of GH and IGF-I, as well as their receptors, in certain areas and cell types in the CNS will further elucidate the influence and potential therapeutic use of these peptides in brain injuries, Alzheimer's disease, learning deficiencies and old age. This is addressed and further discussed in later chapters of this volume.

CONFLICT OF INTEREST

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