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THE INSULIN-LIKE GROWTH FACTORS

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INTRODUCTION

The insulin-like growth factors (IGF-I and IGF-II) are single-chain polypeptides with structural homology to proinsulin. They regulate proliferation and differentiation of a multitude of cell types and are capable of exerting insulin-like metabolic effects. Unlike insulin, they are produced by most tissues of the body and are abundant in the circulation. Thus the IGFs have the potential to act via endocrine as well as autocrine and/or paracrine mechanisms.

The IGFs exert their effects at the cellular level by interacting with the Type-I IGF receptor (IGF-I receptor). They also bind to the Type II/mannose-6-phosphate receptor (IGF-II receptor) and insulin receptors, as well as high affinity binding proteins (IGFBPs). Like the IGFs, the IGFBPs are produced by multiple cell types and have been shown to modulate IGF bioactivity. The recent availability of cDNA probes and antibodies for the IGFs, IGF receptors, and IGFBPs has led to a substantial increase in published studies of IGF physiology. This review attempts to integrate recent information regarding coordinate regulation and function of the IGFs, their receptors, and IGFBPs.

BIOLOGICAL ACTIONS OF IGFS IN VITRO

Effects on Growth and Differentiation

The actions of the IGFs in stimulating growth and differentiation of a wide variety of cell types has been recently reviewed (70). Despite an extensive list of cell types that are responsive to the IGFs, the intracellular mechanisms

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mediating these events are not defined. IGF-I acts as a progression factor in the cell cycle and synergizes with competence factors such as PDGF and FGF. Recent evidence indicates that ras proteins may be required for the mitogenic signal generated by IGF-I late in G1, whereas they are not essential for the action of PDGF or EGF during the Go/G1 transition. In contrast, inhibition of the G protein alpha subunit inhibits the action of PDGF and EGF, but does not affect the IGF-I stimulation of DNA synthesis (72).

IGF-I has been shown to stimulate terminal myogenic differentiation of L6 myoblasts by inducing expression of the myogenin gene. The increase in myogenin mRNA levels in response to IGF-I is blunted by inhibitors of myogenic differentiation, and an oligodeoxyribonucleotide complementary to the amino terminal sequence of myogenin blocks the stimulation of differentiation by the IGFs. The inhibition by the antisense oligomer is specific for differentiation, as none of the other anabolic effects of IGF-I on L6 myoblasts is affected (43, 44).

Autocrine/Paracrine Actions

While the liver is the major source of circulating IGFs, local production is thought to be important in the regulation of growth and differentiation. Induction of differentiation of skeletal muscle cells in vitro by serum withdrawal is associated with increases in IGF-II mRNA levels and IGF-II cell-surface receptors (108, 129). Differentiation of muscle cells is inhibited by an oligonucleotide complementary to the first five codons of IGF-II and addition of IGF-II overcomes the inhibition, thus providing direct evidence that autocrine secretion of IGF-II stimulates myoblast differentiation (45).

Muscle cells secrete several forms of IGFBPs in culture that may modulate the effect of the IGFs on differentiation. The secretion of IGFBP-5 is induced 30-fold within 16 hr of the onset of differentiation in C2 skeletal muscle cells (128), while increased expression of IGF-II during differentiation is associated with decreased expression of IGFBP-2 and IGFBP-5 in mouse myoblasts (41). A shift in the pattern of IGFBP production has also been reported during differentiation of L6 muscle cells (86).

Connective tissue cells have been shown to synthesize IGF-I, IGF-II, and IGFBPs in vitro, and growth hormone (GH), as well as other trophic hormones, regulates their synthesis at the tissue level. IGF-I and -II mRNAs are expressed in human dermal fibroblasts and in connective tissue stromal cells in multiple tissues (70). IGF-I mRNA is expressed in chondrocytes of the epiphyseal growth plate of 28-day old rats. IGF-I mRNA levels are decreased in chondrocytes of hypophysectomized rats and partially restored by GH treatment (96), which supports a role for locally produced IGF-I in mediating the effect of GH on longitudinal bone growth. In addition to GH,

PTH stimulates rapid increases in IGF-I mRNA and peptide levels in osteoblasts, which may be mediated via increases in cAMP production (81). IGF-II synthesis by osteoblasts was constitutive in one study and not regulatable by PTH or other trophic agents (82); however, PTH stimulation of IGF-I and -II release from neonatal mouse calvaria has been reported (69). PTH also stimulates synthesis of IGFBP-4 via a cAMP-dependent mechanism in human osteoblasts (65). Since IGFBP-4 has been shown to inhibit the mitogenic effects of the IGFs in human osteoblasts, the acute effects of PTH on bone resorption could be mediated by the increased production of IGFBP-4 (89). Release of IGF-II from mouse calvaria is stimulated by 1,25-$(OH)_2D_3$ to a similar extent as PTH, but release of IGF-I is inhibited (69). Estrogen also modulates IGF-I, IGFBP-2, and IGFBP-4 levels in osteoblasts, and estrogen stimulation of osteoblast proliferation is blocked by an antibody against IGF-I (21, 42).

Genes for IGF-I and -II are expressed in human and rat ovary, however, cellular sites of synthesis vary between the two species. The granulosa cell is the primary site of IGF-I mRNA expression in the rat (57), whereas IGF-II transcripts are present in theca-interstitial cells, but not granulosa (58). Human luteinized granulosa cells express IGF-II mRNA, but not IGF-I mRNA, while IGF-I peptide is localized in the theca-interstitium (56). Estrogen treatment of immature, hypophysectomized rats increases ovarian levels of IGF-I mRNA while decreasing hepatic levels of IGF-I mRNA and ovarian IGF-II mRNA (57, 58). In the rat ovary, the mRNAs for IGFBP-2, -3, and -4 are localized to interstitial cells, corpora lutea, and atretic granulosa cells, respectively, while IGFBP-5 mRNA is expressed in atretic granulosa cells, corpora lutea, and the surface epithelium (40, 95). IGFBP-1 mRNA is not detectable in the rat ovary; however, it is present in the human ovary (63). Several forms of IGFBPs are secreted by granulosa cells in culture and have been shown to inhibit FSH stimulation of ovarian function (11, 130). Regulation of local production of IGF-I in the uterus may also be important. Estrogen induces IGF-I mRNA levels in the ovariectomized rat uterus, while decreasing IGF-I mRNA abundance in the liver, and IGF-I potentiates the effect of estrogen on DNA synthesis in rat uterus in organ culture (93).

A number of human tumor cell lines have been shown to synthesize IGFs and IGFBPs and to have IGF-I or IGF-II receptors (75). Some of these cell lines grow in the absence of exogenous growth factors, and proliferation in serum-free media can be inhibited by antibodies that bind IGF-I, IGF-II, or the IGF-I receptor. Thus the IGFs may serve as autocrine regulators of tumor growth. Recently, IGF-II has been overexpressed in a breast tumor epithelial cell line (MCF-7), which does not constitutively express IGF-I or -II. IGF-II-expressing clones showed marked morphological changes in anchor-

age-dependent culture and grew in soft agar, while wild-type MCF-7 cells did not show these properties. An antibody against the IGF-I receptor inhibited the growth of IGF-II- expressing clones in serum-free medium (35).

In summary, multiple cell types have been shown to synthesize the IGFs. Furthermore, antibodies against either IGF-I or -II or the IGF-I receptor have been shown to block bioactivity in several systems, thus indicating that autocrine/paracrine growth regulation is a likely possibility. Most of these cell types also secrete IGFBPs, although their specific functions in most systems remain to be determined.

BIOLOGICAL ACTIONS OF IGFS IN VIVO

Effects of IGF Infusion on Growth

Infusion of IGF-I in GH-deficient, mature rats stimulates body weight gain, tibial epiphyseal width, and longitudinal bone growth. However, direct comparison with GH treatment indicates that GH is more potent (70). In one study, infusion of IGF-I or -II to neonatal hypophysectomized rats had no effect on body weight gain, despite normalization of serum IGF-I levels, while GH was stimulatory (52). Although GH treatment causes proportionate organ enlargement, IGF-I treatment consistently results in greater increases in kidney, spleen, and thymus weights (52, 70). These differences may be related to the distinct changes in serum IGFBP profiles that are induced by the two treatments. While IGF-I induces IGFBP-3, GH is required for production of the acid-labile subunit of the large molecular weight ternary complex. This complex is present in GH-treated, but not IGF-I-treated animals (136). This alteration in the carrier complex for IGF-I and -II may alter tissue delivery. It is also possible that these differences reflect direct effects of GH on tissue growth that are independent of its induction of IGF-I.

Transgenic Animal Models

Transgenic mice overexpressing the IGF-I gene have increased body weight gain compared to controls, but the increases are less than those of littermates expressing the GH gene (80). However, in these studies the levels of IGF-I in serum and certain organs were lower in the animals expressing the IGF-I gene compared to the GH-expressing animals. Recently, a transgenic line of mice expressing IGF-I has been crossed to a line of dwarf transgenic mice lacking GH-expressing cells (10). The mice that express IGF-I in the absence of GH grow larger than their GH-deficient transgenic littermates and exhibit weight gain and linear growth similar to that of their nontransgenic siblings. However, analysis of organ growth suggests that GH and IGF-I have some

effects that are distinct. GH appears to be necessary to attain normal liver size, while IGF-I can stimulate brain growth (10).

Renal Function

IGF-I peptide and mRNA are colocalized in the collecting duct epithelium, and IGF-I levels are regulated by GH. Levels of IGF-I peptide, but not IGF-I mRNA, rise in the kidney following unilateral nephrectomy in adult rats, which suggests that changes in translation or in clearance may be occurring (55). Expression of IGF-I and IGF receptor genes following unilateral nephrectomy has recently been shown to be age-dependent, with increases in mRNA levels of IGF-I, IGF-I receptor, and IGF-II receptor observed in remnant kidneys of immature, but not adult rats (91). While kidney cells have been shown to produce IGFBPs (28), their role in regulating the action of IGF-I in the kidney is largely unknown. However, IGF-I and IGFBP-1 are colocalized in the same cell types in multiple nephron segments (62).

Direct infusion of IGF-I increases kidney weight relative to somatic growth in several animal model systems (46, 66, 77) and increases glomerular filtration rate and renal plasma flow in humans (53). This suggests that IGF-I may be a physiologic regulator of renal function. Whether IGF-I has a role as a therapeutic agent in patients with renal insufficiency has not been determined.

MODULATION OF IGF ACTION BY IGFBPS

The IGFBP Family

While attempting to purify IGF-I and -II from serum, investigators found that the IGFs were bound to higher molecular weight carrier proteins. Western ligand blotting, a technique that utilizes the binding properties of the IGFBPs for IGF-I or -II, has been used to obtain molecular weight estimates of several forms of IGFBPs and to determine which forms are present in multiple biological fluids (87, 107). Recently, cDNA cloning has been used to determine the sequences of the genes encoding each of the IGFBPs.

The complete primary structures of six forms of IGFBP have now been determined. They have been designated IGFBP-1 through 6. The mature proteins each consist of 200 to 300 amino acids, with signal sequences of 20 to 40 amino acids. The IGFBPs have 18 cysteine residues in their amino- and carboxyterminal ends whose alignment is conserved, suggesting disulfide bonding may be important in forming the specific high-affinity IGF binding site. An exception is IGFBP-6, which lacks 2 and 4 of the homologous cysteines in the rat and human, respectively. In addition to the 18 cysteines, two additional cysteines are present in rat and human IGFBP-4. IGFBP-1 and

-2 contain an RGD sequence in their carboxyterminal end, which is found in many extracellular matrix proteins and often serves as a recognition sequence for cell surface integrin receptors. Four of the IGFBPs (3 though 6) are glycosylated to varying degrees, which may be important for adherence to cell surfaces.

The first IGFBP to be completely characterized was human IGFBP-1. This protein was purified from human amniotic fluid and decidua. The cDNA sequences of human and rat IGFBP-1 are 58% homologous (87). IGFBP-1 binds IGF-I and -II with approximately equal affinity. At pH 7.0 its affinity constant for IGF-I is fivefold greater than that of the IGF-I receptor. IGFBP-1 mRNA expression is high in human decidua, endometrium, and fetal liver. In the adult rat, IGFBP-1 mRNA expression is highest in the kidney, liver, and decidua (94).

The complete structures of human, rat, and bovine IGFBP-2 have been determined from cDNA sequence analysis (16, 87). The proteins from all three species are approximately 85% homologous. The liver is the predominant site of IGFBP-2 expression in the fetus, although kidney, stomach, lung, and brain also express moderate message levels. Expression is very high in rat brain and higher in fetal than adult tissues (101, 102). The affinity of IGFBP-2 for IGF-II is threefold higher than for IGF-I, but its affinity for both is greater than that of IGFBP-1 at pH 7.4.

IGFBP-3 is the IGF-binding subunit of the 150 kd GH-dependent binding complex that is present in serum (8). IGFBP-3 was originally purified from human plasma, and its primary structure was determined from clones obtained from human liver cDNA libraries. Porcine, rat, and bovine IGFBP-3 have subsequently been cloned. In adult rats, abundant levels of IGFBP-3 are present in the kidney, liver, stomach, placenta, uterus, and ovary (2). IGFBP-3 has a very high affinity for both IGF-I and -II and is the most abundant form of IGFBP in serum. These properties account for the fact that it binds greater than 95% of the IGF-I and -II in the circulation.

IGFBP-4 has been purified from human and rat serum and from media conditioned by a human osteosarcoma cell line (61, 89, 115). Its primary structure has been determined from cDNA sequence analysis (61, 65, 114). The human and rat proteins are 92% homologous. While IGFBP-4 contains a single N-linked glycosylation site, it is generally present in a non-glycosylated form. Higher M_r forms of 28 to 30 kd have been detected in serum and conditioned medium of neuroblastoma cells (22, 61). A single mRNA transcript of 2.6 kb is present in multiple tissues of adult rats, with liver exhibiting the highest expression (95, 114). IGFBP-4 transcripts of 2.0 to 2.3 kb have been detected in human tissues and fetal fibroblasts (19, 61, 65). IGFBP-4 has a high affinity for IGF-I and -II that is nearly equal to that of IGFBP-3 at pH 7.4.

IGFBP-5 has been purified from human and rat sources (3, 7, 113), and its

primary structure has been obtained from clones isolated from rat ovary, human placenta, and human osteosarcoma cDNA libraries (60, 113). The rat and human proteins are 97% conserved, and M_rs of the mature proteins are 28,428 and 28,553, respectively (113). While IGFBP-5 contains no potential N-linked glycosylation sites, the protein migrates as a doublet at 31 to 32 kd on SDS gels under nonreducing conditions. IGFBP-5 has the highest affinity for IGF-I and -II of all the IGFBPs, which is 50-fold greater than that of the IGF-I receptor at pH 7.4.

A single 6.0 kb IGFBP-5 mRNA transcript has been detected in all tissues examined of adult rats. Several tissues have higher levels of mRNA compared to the liver, with the highest expression observed in the kidney (113). A single 6.0 kb transcript is also present in human fetal fibroblasts (19). In addition to the 6.0 kb transcript, a 1.7 to 1.8 kb transcript has been detected in rat ovarian tissue and human osteosarcoma cells (40, 60).

IGFBP-6 has been purified from several human sources, including serum, cerebrospinal fluid, and conditioned media of fibroblasts and osteosarcoma cells (3, 47, 79, 106, 137). The complete primary structure of rat and human IGFBP-6 has been determined from cDNA sequence analysis of clones obtained from rat ovary, human placenta, and human osteosarcoma cells (61, 112). The estimated M_rs of the mature proteins are 22, 847 and 21,461 for the human and rat, respectively. The protein has a 10- to 100-fold higher affinity for IGF-II than for IGF-I (47, 79, 106), which is similar to the affinity of IGFBP-3 for IGF-I. A single transcript of 1.3 kb is abundant in all tissues that have been examined (112).

The human IGFBP-1, 2, 3, 4, and 5 genes are located on chromosomes 7, 2, 7, 17, and 5, respectively (113). The human IGFBP-1, human IGFBP-3, and rat IGFBP-2 genes span 5.2, 8.9, and at least 8 kb, respectively, and have similar structural organization (17, 33, 34). The IGFBP-1 and IGFBP-2 genes contain four exons, while the IGFBP-3 gene contains a fifth exon. The promoter regions of IGFBP-1 and IGFBP-3 each contain a TATA box and a consensus promoter element located upstream from a single mRNA cap site within a CpG island. Liver factor B1 (LFB1) appears to regulate basal IGFBP-1 promoter activity in HepG2 cells by binding the CCAAT promoter element (126). This element is absent from the IGFBP-3 promoter, which contains sequences that could bind Sp1 and AP2. In contrast to IGFBP-1 and -3, the IGFBP-2 gene lacks a TATA box near the transcription start site. Its promoter region contains recognition sequences for several transcription factors including Sp1, ETF, AP-1, AP-2, and LFB1.

Regulation of IGF Bioavailability

The majority of IGF-I and -II in the circulation is associated with a 150 kd complex that is composed of glycosylated IGFBP-3, an 85 kd acid-labile subunit and IGF-I or -II (8). The acid-labile subunit and IGFBP-3 do not interact in the

absence of IGFs. The smaller M_r complex of 40 kd contains IGFBP-1, -2, -4, and -6 and contains most of the unsaturated IGF binding sites in serum (61, 137). IGFBP-5 has not been identified in this complex; however, it has been purified from human serum and very small amounts are detectable in human and bovine serum by immunoblot analysis. Serum levels of IGFBP-3, -2, and -1 in the adult human are approximately 5 ug/ml, 150 ng/ml, and 50 ng/ml, respectively. The half-lives of the IGFs are dependent on their association with IGFBPS. The 10 min half-life of ^{125}I-IGF-I is extended to 30 min when bound to the 40,000 M_r complex and greater than 10 to 15 hr when bound to the 150,000 M_r complex. Radiolabeled des(1-3)IGF-I, which has reduced affinity for IGFBPs, is cleared more rapidly (4, 54).

IGFBPs may also be important in mediating transport of the IGFs from the vascular space. IGFBP-1, -2, -3, and -4 have been shown to cross intact vascular endothelium, either alone or when crosslinked to IGF-I (6, 12a). However, it is not known whether the IGFs can also leave the vasculature in an unbound form. Insulin stimulates transport of IGFBP-1, but not IGFBP-2, across intact capillaries (5), which could be a mechanism by which insulin acutely lowers IGFBP-1 concentrations.

Modulation of IGF Action at the Cellular Level

IGFBPs both inhibit and potentiate the metabolic and mitogenic effects of the IGFs at the cellular level. Addition of purified IGFBP-1, -2, -3, or -4 inhibits binding of IGFs to cell surfaces and thus attenuates IGF bioactivity by sequestering the peptide and preventing receptor interaction (12, 18, 22, 32, 48, 89, 109). At equimolar concentrations, IGFBP-3 is more effective than either IGFBP-2 or IGFBP-1 in blocking cell surface binding (84).

Preincubation of fibroblasts with IGFBP-3 results in potentiation of IGF-I activity, while co-incubation of IGFBP-3 and IGF-I is inhibitory (8, 32). The potentiating effect is associated with increased binding of IGF-I to membrane-associated IGFBP-3 (32). IGFBP-3 released from cell surfaces of fetal fibroblasts has a greater affinity for IGF-I than that remaining on the cell surface (85). Therefore, the potentiating effect of cell-surface associated IGFBP-3 may be the result of a decrease in its binding affinity, which would permit a more favorable equilibrium with the IGF-I receptor. The mechanism by which IGFBP-3 adheres to cell surfaces is unknown, but does not appear to require glycosylation (31).

IGFBP-1 and -2 also potentiate the mitogenic activity of IGF-I; however, IGFBP-1 is more potent than IGFBP-2 and also induces a greater increase in IGF-I binding to the cell surface (16, 24). Two forms of IGFBP-1 have been isolated from amniotic fluid, but only one form potentiates IGF-I activity, and this form associates with cell membranes (87). The mechanism by which cell-surface associated IGFBPs potentiate IGF activity is unknown. Studies

with IGF analogues with altered affinity for IGFBPs and the IGF-I receptor indicate that binding of IGF-I to both IGFBP-1 and the IGF-I receptor is required for potentiation (23).

IGFBP-5 has been shown to potentiate the proliferative actions of IGF-II in osteoblasts (7), while a mixture of IGFBP-5 and -6 enhances IGF-I stimulation of mitogenesis in human osteosarcoma cells (3). Preincubation with the IGFBPs was not necessary to elicit the potentiating effect on IGF activity, and their ability to associate with cell membranes was not determined.

IGFBP-1 is phosphorylated in vitro by human hepatoma (HepG2) and endometrial cells in culture (49, 59). Human amniotic fluid and fetal serum contain both nonphosphorylated and phosphorylated IGFBP-1, whereas HepG2 and decidual cells secrete predominantly phosphorylated isoforms. The phosphorylated IGFBP-1 secreted by HepG2 cells has a sixfold higher affinity for IGF-I than it does after dephosphorylation. IGFBP-3 is also phosphorylated, however, its affinity for IGF-I has not been determined. Dephosphorylated IGFBP-1 purified from amniotic fluid potentiates the effect of IGF-I on DNA synthesis, while the phosphorylated form appears to be inhibitory.

REGULATION OF IGF AND IGFBP SYNTHESIS

Hormonal Regulation

The regulation of circulating concentrations and tissue synthesis of IGF-I by GH is well documented (8, 36). Circulating levels of IGFBP-3 are also regulated by GH; however, this effect is likely mediated by IGF-I since infusion of IGF-I to hypophysectomized rats induces IGFBP-3 (26, 136). While serum levels of IGF-I and IGFBP-3 decrease in a coordinate fashion in hypophysectomized rats, mRNA levels are not coordinately regulated in all tissues. In liver, mRNA levels of IGF-I and IGFBP-3 are decreased to 10 and 50% of controls, respectively, and partially restored with GH treatment. Levels of IGF-I mRNA in the kidney follow a similar pattern; however, kidney IGFBP-3 mRNA levels increase twofold in hypophysectomized rats and are not reduced by GH (2). Synthesis of IGFBP-3 by multiple cell types including chondrocytes, fibroblasts, endometrial and breast carcinoma cells is also stimulated by IGF-I or -II (20, 23, 111). TGF-beta also stimulates IGFBP-3 secretion by human skin fibroblasts (78).

In contrast to IGFBP-3, blood levels of IGFBP-2 are inversely related to GH status. IGFBP-2 levels increase twofold in patients with hypopituitarism (25), and decrease fourfold in lactating dairy cows treated with GH (29). However, GH does not affect IGFBP-2 levels in calorically restricted adults, which suggests that a normal caloric intake is required for GH to suppress IGFBP-2 (25). Infusion of IGF-I in healthy adults increases circulating

IGFBP-2, while patients with non-islet cell tumor hypoglycemia, in which IGF-II is overexpressed, also have elevated IGFBP-2 levels (137). Thus, GH and IGF-I appear to regulate IGFBP-2 in a dis-coordinate manner.

IGFBP-4 secretion is decreased following exposure to IGF-I or -II in fetal fibroblasts and breast carcinoma cells, while insulin at concentrations sufficient to stimulate the IGF-I receptor increases secretion of IGFBP-4 (19, 20). However, this effect appears to be cell-type specific since IGF-I stimulates IGFBP-4 secretion in L6 muscle cells (86). The decrease in IGFBP-4 secretion by fetal fibroblasts that occurs with IGF-I treatment is not accompanied by a decrease in mRNA abundance (19).

There are presently no reports on regulation of IGFBP-5 in vivo. The findings that hepatic abundance of IGFBP-5 mRNA is low and that serum contains very little detectable protein suggest that circulating IGFBP-5 has a minor regulatory role. In contrast, IGFBP-5 that is synthesized by connective tissue cells and localized in extracellular matrix may be important. IGF-I increases IGFBP-5 abundance in fibroblast conditioned media six- to eightfold while causing no change in mRNA levels. In addition, insulin, at doses that should act through the IGF-I receptor, fails to increase IGFBP-5 levels, which indicates that IGF-I is not acting via its receptor to stimulate IGFBP-5 secretion (19). This effect is mediated by a unique mechanism whereby fibroblasts secrete a protease that specifically degrades IGFBP-5. When IGF-I or -II binds to IGFBP-5, degradation is inhibited.

IGFs and IGFBPs have been suggested to play a role in the growth inhibitory effects seen with glucocorticoid excess. Dexamethasone reduces body weight gain and hepatic and tibial IGF-I mRNA abundance in the adult rat without affecting serum IGF-I concentrations, while at the same time increasing IGFBP-1 serum and hepatic mRNA levels (73). Hepatic IGFBP-1 mRNA levels are also increased during dexamethasone-induced growth retardation in fetal rats, however, mRNA levels of IGF-I and -II are not decreased (104). Dexamethasone increases IGFBP-1 mRNA levels by increasing gene transcription (103, 131). Treatment of 4-day old rats with dexamethasone decreases both hepatic IGF-II and IGFBP-2 mRNA levels (102), while treatment of 4-week old rats increases serum and hepatic mRNA levels of IGFBP-3 (74). Secretion of IGFBP-3 and IGFBP-5 by fetal fibroblasts in vitro is decreased by dexamethasone (19).

Nutritional Regulation

Adequate nutrition is essential for IGF-I to promote growth. During undernutrition, attenuation of growth is accompanied by decreases in serum IGF-I that cannot be normalized by GH treatment (27). The decline in IGF-I concentrations during fasting is due to a decrease in binding of GH to its hepatic receptor. These changes are accompanied by decreases in hepatic

IGF-I and GH receptor mRNA abundance (15, 120). A reduction in the rate of IGF-I gene transcription does not account for the decrease in IGF-I mRNA, which suggests that regulation is primarily at the posttranscriptional level (120). Reductions in IGF-I and GH receptor mRNA levels are also observed in nonhepatic tissues, although the two mRNAs are not coordinately regulated in all tissues (15, 71). IGF-I receptor mRNA appears to be up-regulated in several tissues (71). IGF-I treatment has been shown to partially restore the growth rate of malnourished rats (110), thus suggesting that some degree of tissue sensitivity to IGF-I is maintained.

In contrast to the decrease in hepatic GH binding observed during fasting, dietary protein restriction appears to cause GH resistance by a postreceptor mechanism (27). Reductions of IGF-I mRNA levels in liver and skeletal muscle are observed during protein restriction in growing rats (121, 132). However, infusion of IGF-I into protein-restricted rats fails to normalize tail length, weight, or tibial epiphyseal width despite normalization of serum IGF-I levels and enhancement of spleen and kidney growth (127). This suggests that dietary protein restriction causes organ-specific resistance to IGF-I.

Serum levels of IGF-I, IGFBP-3, and IGFBP-2 are relatively constant over the day. In contrast, IGFBP-1 levels show a marked diurnal variation that is inversely related to insulin status and blood glucose levels and is independent of GH secretory status. The regulation of IGFBP-1 in serum has been extensively studied, and a role in glucose counter-regulation has been proposed (67). Administration of an IGF-I/IGFBP-1 complex to rats blocks the hypoglycemic response to exogenous IGF-I, while injection of IGFBP-1 alone increases plasma glucose levels (68). These data suggest that the IGFs may play a role in glucose homeostasis. The effects of insulin on IGFBP-1 appear to be both direct, as well as secondary, to changes in glucose concentrations (116, 124).

Alterations in IGFBP-1 levels due to metabolic status are reflected at the level of hepatic synthesis, although insulin has also been shown to stimulate transport from the vasculature. Hepatic IGFBP-1 production is increased by glucagon, substrate deprivation, and factors that increase cAMP, whereas insulin is inhibitory (67). Insulin decreases IGFBP-1 mRNA levels in rat hepatoma cells by directly decreasing gene transcription (131). Therefore, the regulation of hepatic IGFBP-1 synthesis may be an important determinant of serum IGFBP-1 levels.

Extreme variations in nutrient intake such as fasting also regulate IGFBPs. Plasma levels of IGFBP-3 are decreased by fasting or protein deprivation in growing rats (26), while hepatic mRNA levels of IGFBP-2 and IGFBP-1 are increased by fasting or protein deprivation (94, 101, 102, 121). Maternal fasting increases IGFBP-1 mRNA levels in fetal liver, while having no effect on IGFBP-2 mRNA levels (119). Levels of IGFBP-1 are increased in serum

of neonatal piglets after 48 hr of fasting when analyzed by ligand blot and radio-immunoassay (RIA). In contrast, IGFBP-2 levels are increased by RIA, but decreased by ligand blot analysis, which indicates that an IGFBP-2-specific protease may regulate IGFBP-2 levels in neonatal serum (88).

Developmental Regulation

The IGFs are believed to be important in fetal development, and direct evidence of a role for IGF-II in fetal growth has been demonstrated (38). IGF-I and -II mRNA transcripts are present in virtually all fetal tissues, although their abundance and ontogeny of expression varies among organs. In the fetal rat, IGF-II gene expression is abundant in most tissues, but decreases dramatically at birth in all tissues except the brain. The decrease in IGF-II mRNA levels corresponds to the decrease in circulating levels of IGF-II postnatally. However, this decrease may be unique to the rat, as studies in other species, including humans, have shown that significant serum levels of IGF-II are maintained postnatally. IGF-I transcripts in fetal liver, kidney, and heart are lower in abundance compared with IGF-II and rise progressively with increasing postnatal age, as does serum IGF-I, while expression in fetal lung, muscle, and stomach is higher than it is postnatally (1). In mid-gestation, IGF-I and -II transcripts are found predominately in mesenchymal cells; however, early in organogenesis these transcripts are not always coexpressed, and in the case of IGF-II, not restricted to cells of mesenchymal origin (14).

Levels of IGFBP-1 and -2 are also developmentally regulated, with transcripts for each expressed in a variety of fetal tissues. Expression of both mRNAs is higher in the liver than in other fetal tissues, with abundant levels of IGFBP-2 also present in fetal kidney, brain, and stomach (101). IGFBP-2 transcripts are predominately localized to cells of ectodermal and endodermal origin and expressed in discrete locations in the nervous system during early organogenesis. They are not colocalized with IGF-II mRNA (135). Message and serum levels of IGFBP-1 and -2 fall postnatally in the rat. IGFBP-3 is not present in serum of fetal rats; however, IGFBP-3 mRNA is present in the kidney and liver of 1-day old rats and increases in both organs by week 1. Levels remain constant thereafter, while the levels of hepatic IGF-I mRNA continue to increase up to 8 weeks (2). In contrast to the rat, IGFBP-3 levels are abundant in fetal serum of other species, such as the sheep, cow, and pig, although circulating concentrations are significantly higher in postnatal life.

IGFs and IGFBPs are altered in maternal serum during pregnancy. In the rat, serum IGF-I and hepatic IGF-I mRNA levels are decreased during pregnancy, while serum IGF-II levels are unchanged (37, 39). In the human, serum IGF-I and IGFBP-3 levels determined by RIA increase during pregnancy (8). In contrast, IGFBP-3 is virtually undetectable by ligand blot analysis in pregnant serum of both humans and rodents (37, 39, 50). This decrease has

been attributed to the presence of a protease that would produce fragments that are unable to bind the IGFs, but would react immunologically (37, 51). However, this discrepancy has also been attributed to an alteration in the ability of IGFBP-3 to bind iodinated IGF (122, 123). The exact physiological role that this protease plays in IGF function during pregnancy is not clear. The abundance of liver IGFBP-3 mRNA is not altered during preganancy in the rat, while IGFBP-1 and -4 mRNA levels are increased (39).

THE IGF-I RECEPTOR

The IGF-I receptor mediates most of the biological actions of IGF-I and IGF-II and is present in a wide variety of tissues and cell lines. The affinity (kd) of the IGF-I receptor for IGF-I is approximately 1 nM, 2- to 10-fold lower for IGF-II, and 100- to 500-fold lower for insulin. The IGF-I receptor is an $alpha_2beta_2$ transmembrane glycoprotein that is structurally similar to the insulin receptor and contains tyrosine protein kinase domains in the cytoplasmic portion of the beta subunit. The gene is located on human chromosome 15 and mouse chromosome 7 (125). Promoters of the human and rat IGF-I receptor genes lack TATA and CAAT boxes upstream of a unique transcription start site and contain potential binding sites for several transcription factors (76, 133).

IGF-I Receptor Function

Expression studies of cloned IGF-I and insulin receptor cDNAs indicate that both receptors are capable of mediating short-term metabolic effects, as well as long-term mitogenic effects. The physiological roles of both insulin and IGF-I are therefore likely to be determined by the distribution and abundance of their receptors on the cell surface. However, studies with chimeric receptors containing the extracellular ligand-binding domain of the insulin receptor and the intracellular domain of the IGF-I receptor indicate that while the IGF-I and insulin receptors mediate short-term responses such as glucose transport similarly, receptors containing an IGF-I intracellular domain are more effective in stimulating DNA synthesis than the insulin receptor (64). Thus, insulin and IGF-I receptors have similar, yet distinct, signaling potentials that are defined by the cytoplasmic domain.

The intrinsic tyrosine kinase activity of the IGF-I receptor may mediate the biological actions of IGF-I by phosphorylating endogenous cellular substrates. Several substrates for IGF-I receptor kinases have been identified. The phosphoprotein pp185 is an endogenous substrate of both the IGF-I and insulin receptor kinases. This protein has recently been purified and sequenced and is now designated Insulin Receptor Substrate-1 (IRS-1) (109a, 124a). IRS-1 has been proposed as a multi-site "docking" protein, which upon phosphory-

lation associates with cellular proteins such as phosphatidylinositol 3′ kinase, most likely through src-homology 2 (SH2) domains (20a). The phosphoprotein pp175 is also an endogenous substrate for the IGF-I and insulin receptors in FRTL5 thyroid cells and in L6 myoblasts in which it is associated with the cytoskeleton (9, 30). In undifferentiated L6 cells, stimulation by IGF-I increases IGF-I receptor phosphorylation and pp175 induction, whereas IGF-I-induced phosphorylation of pp175 is almost undetectable after cells differentiate into myotubes. Sequence analysis of pp175 will determine whether this protein is identical to IRS-1, which appears likely. The ability of IGF-I to stimulate mitogenesis, but not glucose transport, is inhibited in cells expressing a kinase-defective insulin receptor. IGF-I mediated phosphorylation of pp170 and pp220 is inhibited in these cells (83). Therefore, while the specific functions of phosphorylated endogenous substrates are unknown, evidence that they may be important mediators of biological activity is accumulating.

IGF-I Insulin Receptor Hybrids

Some tissues and cell types have been shown to express hybrid receptors composed of one insulin receptor alpha and beta subunit and one IGF-I receptor alpha and beta subunit. These receptors may account for the appearance of two beta subunits with different M_r that are frequently observed following IGF-I stimulation. The presence of hybrid receptors was suggested by findings that monoclonal antibodies specific for the insulin receptor immunoprecipitated IGF-I binding activity (117). Evidence that this cross-reactivity is produced by hybrid receptors came from studies in which an antibody specific for the human insulin receptor immunopreciptiated IGF-I binding activity only after expression of the human insulin receptor in rodent cell lines and, conversely, an antibody specific for the human IGF-I receptor acquired the ability to immunoprecipitate insulin-binding activity following expression of human IGF-I receptors in a mouse cell line (118). The hybrid receptor has higher affinity for IGF-I than insulin, and IGF-I is more effective than insulin in inducing a conformational change in the receptor and in activating autophosphorylation (90, 118). Therefore, the hybrid receptor appears to function more as an IGF receptor than as an insulin receptor. The extent to which hybrid receptors constitute the total receptor population has not been defined for most systems. However, in placenta, 70% of the receptors are estimated to be hybrids (117). This suggests that in some tissues these receptors may play an important role in coordinating IGF action.

Developmental Regulation

Levels of IGF-I receptor mRNA decrease in multiple tissues throughout postnatal development in the rat in comparison to fetal levels. In contrast,

IGF-I mRNA levels increase in liver, heart, and kidney postnatally (134). During early organogenesis, mRNA for the IGF-I receptor is widely distributed in fetal tissues and is most prominent in the developing nervous system and muscle. IGF-II mRNA is also widespread and more abundant than IGF-I message, which is relatively localized, suggesting that the actions of IGF-II may be mediated via the IGF-I receptor in the fetus (14). Recently, two cellular patterns of IGF-I receptor gene expression have been described during development of the rat brain (13). Cells of neuroepithelial lineage have a basal level of receptor gene expression that could represent a target for IGFs in CSF and mediate a basic metabolic or trophic function. Superimposed on this constitutive pattern are sets of neurons that show high levels of IGF-I receptor mRNA in conjunction with IGF-I mRNA expression during the period of postnatal differentiation. This suggests that these may represent specific areas of paracrine IGF-I action in the brain parenchyma (13).

THE IGF-II RECEPTOR

Physiological Significance

The IGF-II receptor is identical to the cation-independent mannose-6-phosphate (M6P) receptor, which functions as a lysosomal enzyme targeting protein. This receptor consists of a single polypeptide chain with a large extracellular domain containing separate binding sites for IGF-II and M6P, a very short cytoplasmic region that lacks intrinsic protein kinase activity, and a single transmembrane domain. The majority of the metabolic and mitogenic effects of IGF-II are mediated through the IGF-I or insulin receptors. The limited studies that indicate a role for the IGF-II receptor in mediating the biologic responses of IGF-II are difficult to interpret and have been recently reviewed (98). Therefore, the function of the IGF-II receptor with respect to IGF-II growth promoting activity remains to be elucidated.

Signal Transduction

Recently, the IGF-II receptor has been shown to be coupled to Gi_2, a GTP-binding protein (G protein). In phospholipid vesicles, IGF-II directly couples purified rat IGF-II receptor with porcine Gi_2 (97). In reconstituted vesicles containing clonal human IGF-II receptor and purified G proteins, the stimulatory effect of IGF-II on Gi_2 activation is inhibited by M6P, while Gi_2 is not activated by M6P alone (92). A 14 amino acid segment of the cytoplasmic domain of the IGF-II receptor is able to activate Gi_2, and its activation is blocked by an antibody against this segment (99, 100). Further evidence that IGF-II may signal intracellular events by activating G proteins via the IGF-II receptor comes from the finding that low concentrations of

IGF-II stimulate production of inositol triphosphate in kidney proximal tubular membranes (105). However, this effect is potentiated by M6P, which indicates that the G protein involved may not be analagous to Gi_2. The relationship of this G protein signaling mechanism to the IGF-related growth response has not been defined.

SUMMARY

The recent availablity of reagents to study the IGFs, their receptors, and binding proteins has led to an explosive growth in the study of IGF physiology. However, most studies to date have been descriptive, and studies delineating mechanisms of action are limited. It is apparent that most organ systems synthesize several components of the IGF system necessary for IGF to function in an autocrine or paracrine fashion and that regulation of this system occurs at the local level. However, the relative importance of locally produced IGF vs circulating IGF remains unclear. The mechanisms by which the IGFBPs modulate IGF activity are crucial to understanding this system, and identification of specific roles for each of these proteins will be required. Of critical importance is the identity of the intracellular signal transduction system by which the IGF receptor mediates the effects of the IGFs, and the delineation of mechanisms by which the IGFBPs interact with the receptor at the cellular level. It is also of interest to determine what role, if any, the IGF-II receptor plays in mediating the growth-promoting effects of the IGFs.

The ubiquitous distribution of the IGFs, IGFBPs, and IGF receptors indicates that they may play a role in the regulation of coordinate growth amoung several tissues and cell types. Understanding the mechanisms by which these components interact to coordinate growth responses between different cell types should greatly enhance our understanding of normal growth and development.

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