Regulation of β-Cell Mass by Hormones and Growth Factors

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Substantial new information has accumulated on molecular mechanisms of pancreas development, regulation of β-cell gene expression, and the role of growth factors in the differentiation, growth, and regeneration of β-cells. The present review focuses on some recent studies on the mechanism of action of cytokines such as growth hormone (GH) and prolactin (PRL) in β-cell proliferation and gene expression—in particular, the role of signal transducers and activators of transcription (STAT) proteins. The implication of the discovery of suppressors of cytokine signaling (SOCS) proteins for the interaction between stimulatory and inhibitory cytokines, including GH, PRL, leptin, and the proinflammatory cytokines interleukin-1 and interferon-γ, in β-cell survival is not yet clear. Recent studies indicate a role of cell adhesion molecules and the delta-like protein preadipocyte factor 1/fetal antigen 1 (Pref-1/FA-1) in cytokine-induced β-cell growth and development. Surprisingly, glucagon-like peptide-1 (GLP-1) was recently found to stimulate not only insulin secretion but also β-cell replication and differentiation, which may present a new perspective in treatment of type 2 diabetes. Together with the intriguing reports on positive effects of insulin on both β-cell growth and function, a picture is emerging of an integrated network of signaling events acting in concert to control β-cell mass adaptation to insulin demand. *Diabetes* 50 (Suppl. 1):S25–S29, 2001

In contrast to what was believed, the endocrine pancreas is subjected to dynamic changes in response to variations in demand for insulin. The β-cell population is kept in delicate balance by β-cell formation (by neogenesis and proliferation) and β-cell death (by senescence, apoptosis, and necrosis). β-Cell mass is closely correlated with body weight, obesity, and insulin demand. The factors involved include nutrients such as glucose, amino acids, and free fatty acids (FFAs) and hormones such as insulin, IGF-I, IGF-II, glucagon-like peptide-1 (GLP-1), glucagon, gastroinhibitory peptide (GIP), gastrin, cholecystokinin (CCK), growth hormone (GH), prolactin (PRL), placental lactogen (PL), and leptin (1). These and other factors that have been shown to either suppress or stimulate β-cell growth, survival, differentiation, or insulin secretion are compiled in Table 1. Because several of these will be discussed in other articles in this issue, the present review will focus mainly on the molecular mechanisms of GH and PRL effects in the β-cell and their interaction with some of the cytokines and hormones listed in Table 1.

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FA-1, fetal antigen 1; FFA, free fatty acid; GH, growth hormone; GHR, GH receptor; GLP-1, glucagon-like peptide-1; GIP, gastroinhibitory peptide; hGH, human GH; IRF, interferon regulatory factor; IRS, insulin receptor substrate; PI, phosphatidylinositol; PL, placental lactogen; Pref-1, preadipocyte factor 1; PRL, prolactin; PRLR, PRL receptor; SOCS, suppressors of cytokine signaling; STAT, signal transducers and activators of transcription.

EFFECTS OF GH AND PRL IN β-CELLS

Early experiments in rats undergoing transplantation with a GH- and PRL-producing tumor suggested that these hormones are able to stimulate β-cell proliferation. However, a direct stimulatory effect could be documented only when it became possible to maintain isolated pancreatic islets in culture for prolonged periods. Thus, it has been shown that GH, PRL, and PL can stimulate β-cell proliferation, glucose-induced insulin release, and insulin gene expression and biosynthesis in fetal, newborn, and adult rat islets in culture (2–4). The expression of GH receptor (GHR) and PRL receptor (PRLR) mRNA is markedly increased in the pancreas from pregnant rats (5) and at the protein level for PRLR (2). In the liver and other tissues, GH induces the expression of IGF-I, thought to mediate the growth-promoting effects of GH. We found, however, no indication for either expression of IGF-I or its mediation of the mitogenic effect of GH in isolated newborn rat islets (6), although increased expression has been demonstrated in ductal cells in the regenerating pancreas (7), and a mitogenic effect was reported in INS-1 cells (8).

GH AND PRL SIGNALING PATHWAYS

Knowledge on the signal transduction mechanisms of GHR and PRLR is accumulating exponentially. We and others identified distinct domains in the cytoplasmic part of the GHR involved in different biologic effects. Thus, a proline-rich domain, box 1, seems to be sufficient, at least in some cell lines, for mitogenic signaling via the activation of the tyrosine kinase JAK2, whereas a COOH-terminal domain has an additional indispensable function in the stimulation of the transcription of certain GH-regulated genes, e.g., insulin and somatostatin (9–11). Recently, a new family of transcription factors, signal transducers and activators of transcription (STAT), was found to be activated by cytokines including GH

TABLE 1
List of factors that inhibit or stimulate β-cell growth and/or function

	Inhibitors	Stimulators
Metabolites	Glucose, FFA	Glucose, FFA, amino acids
Cytokines	IL-1, IFN-γ, TNF-α, leptin	GH, PRL, PL
IGF family	IGF-I	IGF-I, IGF-II, insulin
EGF family		TGF-α, betacellulin, HB-EGF
FGF family		aFGF
VEGF/PDGF family		VEGF, PDGF
HGF family	HGF	HGF
Glucagon family		GLP-1, GIP, glucagon
Somatostatin family	Somatostatin	
CGRP family	IAPP/amylin	
Gastrin family		Gastrin, CCK
TGF-β family	TGF-β, follistatin	Activin A
Neurotrophins		NGF, NT-3
Neurotransmitters	(Nor)epinephrine	Acetylcholine
Delta-like proteins		Pref-1/FA-1
Lectins		reg/INGAP/ PSP/PTP
Adhesion molecules		Integrin α6β1
Drugs	Diazoxide	Nicotinamide, SU
Toxins	Streptozotocin, alloxan	

See Nielsen and Serup (1) for further details. aFGF, acidic fibroblast growth factor; HB-EGF, heparin-binding EGF-like protein; HGF, hepatocyte growth factor/scatter factor; IAPP, islet amyloid polypeptide; IFN, interferon; IL, interleukin; INGAP, islet neogenesis-associated peptide; NGF, nerve growth factor; NT-3, neurotrophin-3; PDGF, platelet-derived growth factor; PSP, pancreatic stone protein; PTP, pancreatic thread protein; SU, sulfonylurea; TGF, transforming growth factor; TNF, tumor necrosis factor; VEGF, vascular endothelial growth factor.

and PRL, which activate STAT1, -3, and -5. Whereas STAT1 and -3 are activated directly by JAK2, STAT5 activation requires binding to phosphotyrosine residues in the COOH-terminal part of the GHR to be phosphorylated and activated (12). In addition, to activate the JAK/STAT pathway, GH and PRL induce a number of signaling events, including the mitogen-activated protein kinase cascade, insulin receptor substrate (IRS)-1 and -2, phosphatidylinositol (PI) 3-kinase, protein kinase C, and rises in intracellular Ca^{2+} (13). JAK2 is rapidly tyrosine phosphorylated in response to GH and PRL in INS-1 cells, and inhibition of tyrosine kinase activity was shown to abolish their mitogenic effect (14). Stimulation of INS-1 cell proliferation by IGF-I has been suggested to involve IRS-mediated induction of PI 3-kinase activity (8). However, the mitogenic effects of IGF-I and human GH (hGH) were found to be additive (15), indicating that GH and PRL use other pathway(s) to induce proliferation in these cells (16).

We have recently demonstrated that GH and PRL activate STAT5a and -5b in insulin-producing cells (17,18). This is supported by data from Stout et al. (19) and Svensson et al. (20), who showed GH- and PRL-induced nuclear translocation of STAT5a and -5b in INS-1 cells and in cultured rat islets. Furthermore, these authors recently found increased nuclear translocation of STAT5b and -5a during pregnancy and lactation, respectively (20). We have identified STAT5 binding elements in the rat insulin-1 promoter as well as in one of the PRLR promoters, required for full induction of these genes by GH and PRL (17,18). The STAT5 binding element of the rat insulin-1 promoter conferred GH and PRL responsiveness to a heterologous promoter in RIN-5AH cells (17).

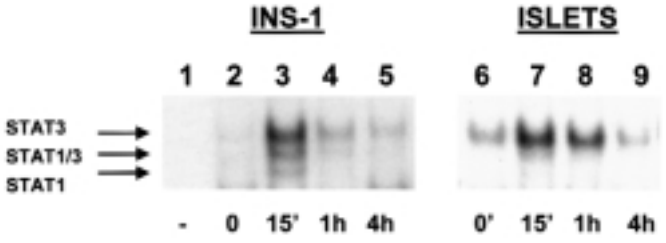

FIG. 1. The kinetics of hGH-induced activation of STAT1 and STAT3 in INS-1 cells and in newborn rat islets. Gel retardation analysis was performed as described (17) using nuclear extracts isolated from INS-1 cells (*lanes 2–5*) or newborn rat islets (*lanes 6–9*) that had been cultured in RPMI 1640 medium containing 0.5% serum and incubated in the absence (*lanes 2* and *6*) or presence of hGH (500 ng/ml) for 15 min (*lanes 3* and *7*), 1 h (*lanes 4* and *8*), or 4 h (*lanes 5* and *9*). The radiolabeled double-stranded oligonucleotide probe M67 containing the optimized STAT1/STAT3 binding site from the c-*fos* promoter was incubated without (*lane 1*) or with (*lanes 2–9*) nuclear extracts. Free and bound probes were separated by nondenaturing PAGE and visualized by autoradiography. Arrows indicate complexes containing STAT3 homodimers (upper arrow), STAT3/STAT1 heterodimers (middle arrow), and STAT1 homodimers (lower arrow). The autoradiograph shown is representative of three independent experiments.

In addition to STAT5, we have demonstrated that STAT1 and -3 are activated by GH and PRL in the RIN-5AH cells (17). To determine if GH and PRL activate STAT1 and -3 in cultured newborn rat islets and in INS-1 cells, gel retardation analysis was performed (Fig. 1). STAT3 and, to a lesser extent, STAT1 DNA binding was increased in INS-1 cells treated for 15 min with hGH, which activates GH as well as PRL receptors in rodents (Fig. 1, *lane 3*). Whereas STAT3 was also found to be activated by hGH in cultured newborn rat islets, STAT1 activation was hardly detectable in these cells (Fig. 1, *lane 7*). The activation of STAT1 and -3 was transient, with their DNA binding markedly decreasing after 1 h of hGH treatment (Fig. 1, *lanes 4* and *8*) and returning to basal within 4 h (Fig. 1, *lanes 5* and *9*). In contrast, hGH was found to induce long-term activation of STAT5 in INS-1 cells as well as in newborn rat islets (20a). These results indicate that STAT5 is the most important STAT protein in GH and PRL signaling in insulin-producing cells.

STAT PROTEINS IN CELL REPLICATION AND SURVIVAL

STAT5 is a potent mediator of mitogenic signals in lymphoid cells (21), and mice deficient in STAT-5a and -5b show defective T-cell proliferation, lacking expression of genes controlling cell cycle progression, i.e., cyclins and cyclin-dependent kinases (22). GH and PRL enhance expression of cyclin D1 in human breast cancer cells (23), whereas PRL induces expression of cyclin D2 and D3 during promotion of G_1/S transition of rat Nb2 cells that depend on PRL for proliferation (24). By using a dominant-negative STAT5 mutant, we show that STAT5 activation is essential for the mitogenic effect of GH and PRL in insulin-producing cells (20a).

STAT5 has also been implicated in the regulation of cell death and survival. Thus, STAT5 deficiency increased the susceptibility of cytokine-dependent cells to apoptosis (25). The antiapoptotic effect of STAT5 has been explained by induction of the *Bcl-xL* gene, which contains a STAT5 responsive element (25). Accordingly, antiapoptotic effects of GH and PRL in Nb2 cells have been correlated with increased Bcl-2 expression and decreased Bax expression (26). Because exogenous expression of Bcl-2 and Bcl-xL proteins has been reported to protect rodent as well as human β-cells from apoptosis (27,28), it seems likely that GH and PRL prevent proinflammatory cytokine and T-cell–induced apoptosis of β-cells by STAT5-mediated regulation of *Bcl-2*–related genes. In addition, STAT5 inhibits STAT1-induced expression of interferon regulatory factor (IRF)-1 in Nb2 cells (29). In accordance with this, GH and PRL appeared to decrease DNA binding to the STAT1-regulated element of the IRF-1 promoter in INS-1 cells (30). Thus, GH and PRL may protect β-cells against cytotoxic cytokines via activation of STAT5 (31).

Leptin, which appears to play an important role in obesity and type 2 diabetes, is a member of the cytokine family, and its receptor is expressed in β-cells, in which it inhibits insulin release and gene transcription (32). On the other hand, leptin has also been shown to protect β-cells against FFA-induced apoptosis (32a). Like GH and PRL, leptin signals via the JAK/STAT pathway, and it remains to be determined by which mechanism the opposite effects are exerted on the insulin gene transcription (12).

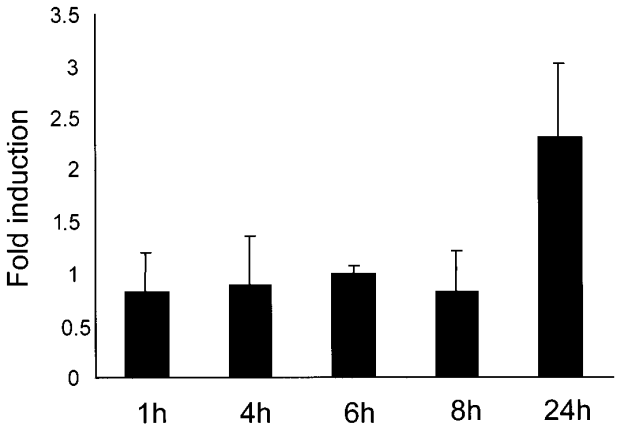

FIG. 2. The effect of duration of GH stimulation on the proliferation of neonatal rat β-cells. Monolayer cultures of neonatal islet cells were prepared essentially as previously described (6). After establishment of the hGH-promoted monolayer, the cells were cultured for 24 h in the absence or presence of 0.5 μg/ml hGH for the number of hours indicated. A total of 10 μmol/l BrdU was present during 24 h. The cells were double-stained for BrdU and insulin as described previously (6). The results are expressed as the fold-increase in number of BrdU-labeled β-cells compared with controls (mean ± SD, *n* = 2–5).

SOCS PROTEINS IN CYTOKINE SIGNALING

Recently, a new family of genes transiently induced by cytokines, called suppressors of cytokine signaling (SOCS), has been discovered. A unique feature of these proteins is that they act as inhibitors of the cytokine receptor signaling. As of now, eight members are known, i.e., cytokine-inducible SH2-containing protein and SOCS1–7. We found that GH preferentially induces the expression of SOCS-3 and that SOCS-3 inhibits GH-stimulated gene transcription and STAT5 activation (33,34). Because STAT binding elements have been found in the promoters of at least some of the SOCS genes, it is conceivable that they may participate in a complex negative cross-talk among several cytokine receptors (35). The role of the SOCS proteins in GH- and PRL-mediated β-cell replication and resistance to proinflammatory cytokines is currently being investigated.

GH- AND PRL-REGULATED β-CELL GENES

Although the mitogenic effect of GH and PRL in β-cells is direct, it still may require the transcriptional activation of genes. To determine whether a single signaling event such as STAT5 activation is sufficient for triggering the mitotic activity, we exposed neonatal rat islet cells to hGH for various time intervals. Exposure for 1, 4, 6, or 8 h did not lead to progression into S phase after 24 h (Fig. 2). Thus, maintenance of the stimulus for >8 h is necessary for the mitotic response, suggesting that a sustained or repeated activation of signaling molecules, STAT5, and transcription of several genes including cyclins are involved. To understand the molecular mechanisms, it is thus crucial to identify these genes.

Besides stimulation of the insulin and PRLR gene expression, GH and PRL have been shown to upregulate a novel islet protein (preadipocyte factor 1 [Pref-1]), which was recently cloned from the 3T3-L1 preadipocyte-like cell line (36). Pref-1 is a transmembrane protein containing six epidermal growth factor (EGF)-like motifs and can be converted by proteolytic cleavage into a soluble form, which was recently isolated from human amniotic fluid as fetal antigen 1 (FA-1). It was shown to be expressed in most epithelial cells in the early embryonic pancreas but became confined to the β-cells close to term in both humans and rats (36). In the adult, it is expressed only in β-cells, adrenals, and somatotrophs. The expression level is low in adult β-cells but markedly upregulated in pregnancy (36). The function of Pref-1/FA-1 remains to be elucidated, but because it shares some homology with the Notch ligands Delta and Jagged, it may play a role in maintaining cells in a proliferative state.

Because GH and PRL promote attachment and spreading of islet cells in culture, we have studied changes in the expression of cell adhesion molecules (37). We found that GH and PRL stimulate the expression of the integrin α6β1, which may be a prerequisite for replication because desintegrin peptides significantly reduced the mitogenic effect of GH and PRL in primary rat islet cells (A.M., M.N. Lindberg, J.H.N., unpublished data). Thus, intracellular signals mediated by the integrins may contribute to the mitogenic effect of these hormones in the β-cell, although this needs further investigation.

GLP-1 IN β-CELL GROWTH AND DEVELOPMENT

Among the intestinal factors involved in glucose-regulated insulin release, the so-called incretins, GIP and Glp-1, are so far the most prominent members. Thus, treatment of type 2 diabetic patients with Glp-1 has been shown to normalize glucose tolerance. This effect has mainly been ascribed to the potentiating effect on glucose-stimulated insulin release. Recently, however, it was found that Glp-1 can stimulate proliferation of both INS-1 cells and normal rat islet cells, as evaluated by incorporation of tritiated thymidine (38). By

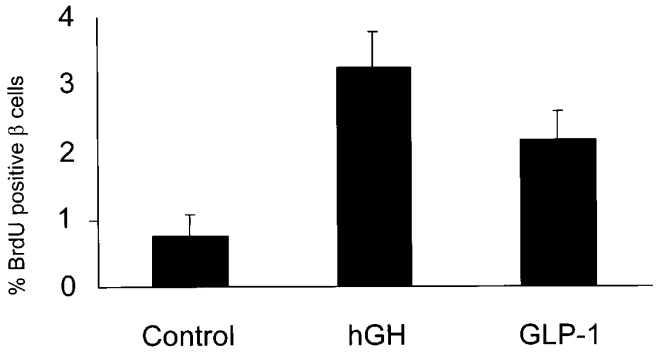

FIG. 3. Effect of Glp-1 and hGH on β-cell proliferation in neonatal rat islet cells. Monolayer cultures of neonatal islet cells were prepared essentially as previously described (6). After establishment of the hGH-promoted monolayer, the cells were cultured for 24 h in the absence or presence of 0.5 μg/ml hGH or 100 nmol/l glucagon-like peptide 1 (GLP-1). Ninety minutes before fixation, 10 μmol/l BrdU was added. The cells were double-stained for BrdU and insulin as described previously (6). The results are expressed as BrdU-labeled β-cells in percent of the total number of β-cells (mean ± SE, n = 4). A total of 1,000 cells were counted for each preparation.

using another experimental set-up in which we measured the incorporation of 5-bromo-2-deoxyuridine (BrdU) into neonatal rat islet cells, we found a 3-fold increase in the number of $BrdU^+$ β-cells compared with a 4.5-fold increase in the presence of hGH (Fig. 3). The effects were additive, suggesting that different pathways are involved (data not shown). Glp-1 and the analog exendin-4 have also been reported to stimulate the expression of Pdx-1 in INS-1 cells (38) and to induce expression of insulin and glucagon in the pancreatic tumor cell line AR42J (39), suggesting that Glp-1 is involved in the differentiation of islet stem cells to endocrine cells. In this context, it is interesting that we demonstrated expression of the prohormone converting enzyme proprotein convertase 1/3 in the glucagon-containing cells in the embryonic rat pancreas, suggesting that proglucagon may be processed to Glp-1 during pancreas development (40). Thus, Glp-1 may promote β-cell differentiation and growth via a paracrine effect at this stage, in contrast to the postnatal period when Glp-1 is produced only in the intestinal L-cells.

CONCERTED ACTION OF β-CELL GROWTH FACTORS

The intriguing finding that insulin receptors in β-cells mediate stimulatory signals on both insulin secretion and gene transcription as well as cell growth and survival suggests that insulin as well as IGF-I and IGF-II contribute to the regulation of β-cell growth, function, and survival. The often contradictory positive and negative cross-talks between the signaling pathways for receptors of various classes, i.e., tyrosine kinase receptors, G-protein–coupled receptors, cytokine receptors, and adhesion molecules, have further added to the complexity of this regulation. In Fig. 4, some of the pathways involved in β-cell regulation are depicted. A better understanding of the integration of the signaling events in time and space of this "neural" network will hopefully lead to identification of their roles in the adaptation of the β-cell mass to insulin demand under various physiological conditions. The ultimate goal is prevention and cure of diabetes by protection or regeneration of endogenous β-cells or by transplantation of β-cells derived from endogenous stem cells or created by genetic modification of other cell types.

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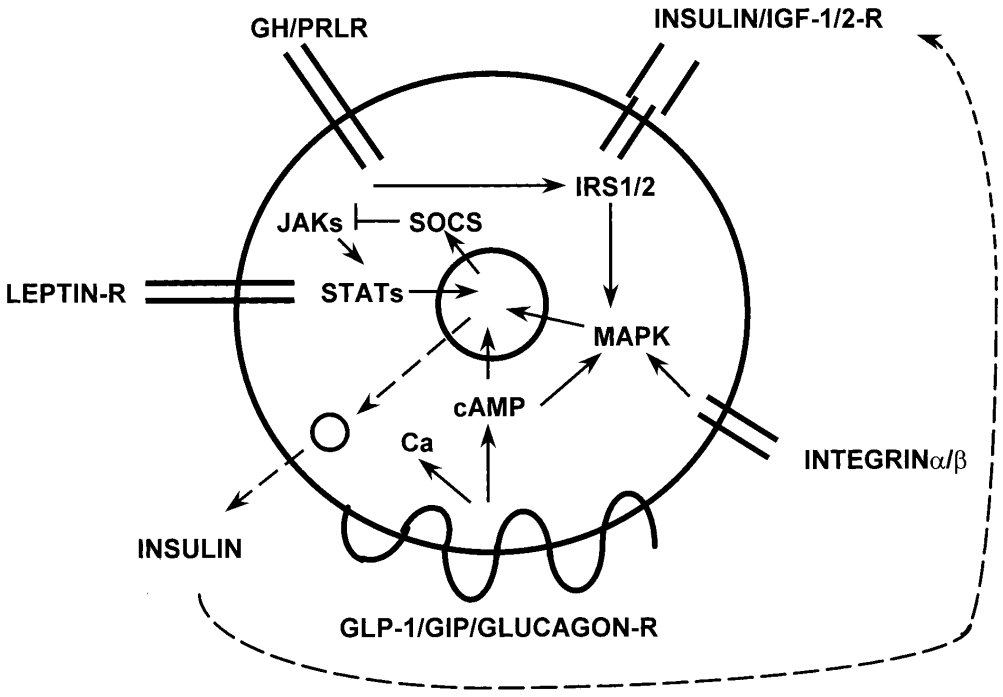

FIG. 4. Signaling pathways supposedly involved in β-cell replication and survival. For explanations, see text. GLP-1, glucagon-like peptide 1; MAPK, mitogen-activated protein kinase; R, receptor.

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