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Growth hormone signaling in pancreatic β -cells – Calcium handling regulated by growth hormone

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ABSTRACT

Deficiency in insulin secretion is a fundamental part in the pathogenesis of all forms of diabetes, determined by impaired secretory function and inadequate β -cell mass. Growth hormone (GH) is a multifunctional hormone, involving in cellular metabolism, mitogenesis and differentiation. In pancreatic islets, GH is involved in maintaining β -cell mass, stimulating islet hormone production and insulin secretion, and, therefore, plays a role in maintaining normal insulin sensitivity and glucose homeostasis. The intracellular events that convey the GH signal into various cellular responses remain incompletely understood. In this review, we discuss GH signaling in the β -cells, with emphasis on Ca^{2+} handling and insulin secretion regulated by human GH (hGH). hGH-stimulated rise in $[\text{Ca}^{2+}]_i$ is dependent on extracellular Ca^{2+} and is mediated by Ca^{2+} -induced Ca^{2+} release (CICR) in the β -cell. This process is triggered by hGH-stimulated activation of the non-receptor tyrosine kinases JAK2 and c-Src, which causes tyrosine phosphorylation of RyRs, resulting in sensitization of CICR. The rise in $[\text{Ca}^{2+}]_i$ elicited by hGH is associated with an enhanced insulin secretion, effects that are mediated mainly through the prolactin receptor. These mechanisms indicate that a rise in $[\text{Ca}^{2+}]_i$ elicited by activation of PRLR is JAK2-dependent and is associated with enhanced insulin secretion. In contrast, GH receptor-mediated increase in $[\text{Ca}^{2+}]_i$ is JAK2-independent and is dissociated from insulin secretion.

Pancreatic β -cells are unique in their ability to synthesize and secrete insulin, which keeps glucose levels within a physiological range. The capacity of the β -cells to respond to elevated blood glucose with increased insulin secretion depends on a sophisticated regulation of the insulin secretory machinery by individual cells. Failure of the capacity of the β -cell is a fundamental part of the pathogenesis of all forms of diabetes. Although insulin resistance is one of the major contributors, diabetes develops only when β -cells fail to compensate for increased insulin demand (Weir et al., 2001). β -Cell dysfunction in diabetes involves a number of impairments, including decreased secretory response to glucose (Bell et al., 2001; Gloyn et al., 2003; Abdul-Ghani et al., 2006; Banhegyi et al., 2007; Goodarzi et al., 2007; Mizuno et al., 2007), impeded pulsatile insulin release (Porksen 2002) and inefficient proinsulin processing to insulin (Kahn et al., 1995; Kahn et al., 1997; Loos et al., 2007).

Inadequacy of the pancreatic β -cell results from a combination of impaired secretory function and inadequate β -cell mass. The total β -cell mass is a major determinant of the amount of insulin that can be secreted by the pancreas, and might become rate-limiting in long-term demand invoked on insulin secretion, such as in obesity and pregnancy. The amount of insulin secreted depends on long-term adaptations of the total β -cell mass, which is determined by a balance between islet β -cell neogenesis and apoptosis. The pancreatic β -cell has an estimated life-span of approximately 60 days (Bonner-Weir 2000). A slow turnover of β -cells remains in adult life, and their proliferative activity decreases with increasing age when diabetes is also becoming more prevalent. About 0.5 % of the adult β -cell population is

undergoing replication, which is usually balanced by a small portion of β -cells entering into apoptosis (Bonner-Weir 2000; Bonner-Weir 2000). Adult β -cell proliferation can, however, be enhanced. Healthy β -cells undergo proliferation in response to increased demand, for example, in obesity or pregnancy (Bonner-Weir 2000; Lingohr et al., 2002). The ability of the pancreatic β -cell to expand its proliferative capacity in response to an increased insulin demand may be of critical regulatory significance for the development of diabetes. Diabetic patients, in particular those suffering from type 1 diabetes, but also type 2 diabetes, exhibit a reduced β -cell mass, possibly due to increased rates of apoptosis (Sjoholm 1996; Pick et al., 1998; Lingohr et al., 2002; Sesti 2002; Dickson et al., 2004). In different animal models, a defect in β -cell regeneration seems to be of central importance in the development of glucose intolerance (Sjoholm 1996; Liu et al., 2004). Despite intensive studies, the molecular mechanisms causing the disorder still remain elusive. To achieve a more complete understanding of etiology and pathogenesis of diabetes, further elucidation of factors governing insulin production and proliferation of the β -cell is clearly warranted.

GH regulates proliferation and function of the pancreatic β -cell

GH is a multi-functional hormone, whose functions at the cellular level can be divided into three categories, *viz.* metabolism, mitogenesis and differentiation. GH levels in the blood rise during pregnancy and lactation, suggesting that elevated circulating levels of the hormone are associated with and responsible for the

expansion of the β -cell mass that occurs under these conditions (Brelje et al., 1991).

Maintaining islet β -cell mass and adequate insulin secretion to meet metabolic demands is crucial to avoid glucose intolerance and the development of type 2 diabetes. GH is closely involved in maintaining pancreatic islet size, enhancing β -cell replication and differentiation, stimulating insulin gene expression and hormone production, insulin secretion and maintaining normal glucose homeostasis. (Nielsen et al., 1989; Billestrup et al., 1991; Rhodes 2000; Sjöholm et al., 2000; Fernandez et al., 2001; Nielsen et al., 2001; Okuda et al., 2001; Yakar et al., 2001; Lu et al., 2004) GH overexpression *in vivo* increased pancreatic islet number and volume in transgenic mice (Parsons et al., 1995), while disruption of GH signaling by knockout of the GH receptor gene ($GHR^{-/-}$) in mice resulted in hypoglycemia and hypoinsulinemia, associated with diminished pancreatic islet size and β -cell mass (Liu et al., 2004). The average size of the islets found in $GHR^{-/-}$ mice was only one third of that in wild type littermates with 4.5-fold reduction in total β -cell mass (Liu et al., 2004). Adult $GHR^{-/-}$ mice exhibited significant decrease in glycemia and insulin levels, as well as β -cell insulin mRNA accumulation. Conversely, β -cell mass was substantially expanded in rats bearing GH-secreting tumors (Garay et al., 1971; Parsons et al., 1983). Interestingly, in this animal model, the increased β -cell mass occurred without concomitant hyperglycemia, suggesting a direct stimulatory effect of GH on β -cell mitogenesis. Similarly, β -cell growth is enhanced in patients with acromegaly (Hellman et al., 1961), an influence that may be due to a combination of direct β -cell

trophic effects and compensatory growth of the β -cell to meet an increased insulin demand due to insulin resistance under the circumstances.

GH and the biologically related lactogenic peptides prolactin and placental lactogen have been extensively investigated with regard to effects on β -cell proliferation. GH has been reported to stimulate the *in vitro* replication of fetal, neonatal and adult rat β -cells (Hellerstrom et al., 1991). In most of these studies there was also a stimulatory effect of GH on the insulin content and/or secretion and the majority of effects were mimicked by prolactin and placental lactogen (Hellerstrom et al., 1991; Zhang et al., 2006). Mutation of GHR was found to abolish GH-stimulated insulin production (Moldrup et al., 1991).

Because GH in many other tissues appears to elicit its biological activities by inducing local production of insulin-like growth factors (IGFs) in target cells, the issue of whether a similar paracrine pathway operates also in islets has been addressed. Previous reports have shown that this probably is not the case in the β -cell (Billestrup et al., 1991). Recent studies in GHR-deficient mice revealed that the reduced β -cell mass in the transgenic model was restored, associated with an improved insulin secretion by pancreatic islet-specific overexpression of IGF-1 on the GHR^{-/-} background (Guo et al., 2005). Since GHR gene deficiency causes a concurrent decrease in the production of IGF-1, which also plays a role in islet cell growth, insulin secretion, and maintaining insulin sensitivity (Fernandez et al., 2001; Yakar et al., 2001; Lu et al., 2004), the result observed in GHR^{-/-} mice suggests that

IGF-1 deficiency may be involved in the mechanisms underlying the reduced β -cell mass and function in this animal model.

GH receptor signaling pathways

The effects of GH are mediated through its receptors, which are expressed in most tissues, including β -cells (Moldrup et al., 1990; Nielsen et al., 1990). The GH receptor was the first identified member in the cytokine receptor superfamily, which includes the prolactin (PRL) receptor and receptors for other cytokines. The common nature of the family of the receptors is that they do not contain intrinsic kinase activity. Activation of the receptors, such as GH receptors, results in association and activation of the cytoplasmic tyrosine kinases (Dominici et al., 2005). The Janus family of tyrosine kinases (JAK) is believed to be the major non-receptor tyrosine kinases required for the initiation of GH signal transduction upon ligand binding to the receptor (Foster et al., 1988; Lis et al., 1993; Silva et al., 1993). Among the JAK members (JAK1-3 and Tyk2), the predominant JAK kinase utilized in GH signaling is JAK2 (Lis et al., 1993; Waters et al., 2006), although GH has also been shown to induce tyrosine phosphorylation of JAK1 (Smit et al., 1996) and JAK3 (Johnston et al., 1994). JAK proteins have a molecular mass of approximately 130 kDa. Knockout of JAK2 in mice is embryonic lethal (Neubauer et al., 1998; Parganas et al., 1998), suggesting an important role of the kinase in early development. The interaction site of JAK2 with the GH receptor is at Box1 region, which consists of eight residues (Argetsinger et al., 1993; Carter-Su et al., 1994; Flores-Morales et al., 2006) crucial

for JAK2 activation. Activation of JAK2 occurs as a consequence of ligand-induced aggregation of the receptor and the kinase (Argetsinger et al., 1993; Stofega et al., 2000). The activated JAK2 in turn autophosphorylates JAK2 itself (Sandberg et al., 2005) and the intracellular domain of the GH receptor. Tyr1007 and Tyr1008 within the JAK2 molecule are sites of the autophosphorylation. Mutation of these sites essentially eliminates kinase activity (Feng et al., 1997). The phosphorylated receptor and JAK2 provide docking sites for a variety of signaling molecules that contain SH₂ or other phosphotyrosine-binding (PTB) motifs. The major signaling pathways activated following JAK2 activation are the insulin receptor substrate (IRS)-phosphatidylinositol-3 kinase (PI₃K) pathway, which is mainly involved in GH-mediated actions on carbohydrate and lipid metabolism, the Ras-MAP kinase pathway, and the signal transducer and activator of transcription (STAT) pathway (Moutoussamy et al., 1998; Stofega et al., 2000), which are crucial in gene transcription regulated by GH (Dominici et al., 2005). In addition to JAK2, members of another non-receptor tyrosine kinase family, Src (*e.g.* c-Src and c-Fyn), have been shown to be activated and involved in GH- or PRL-induced cellular events (Clevenger et al., 1994; Berlanga et al., 1995; al-Sakkaf et al., 1997; Zhu et al., 1998; Al-Sakkaf et al., 2000). Among nine members in Src kinase family identified, c-Src was the first cellular proto-oncogene or homologue form of a viral oncogene (v-Src) discovered and the activity of the kinase is regulated by phosphorylation (Bjorge et al., 2000). Termination of GH signaling involves suppressor of cytokine signaling (SOCS) proteins (Tam et al., 2001; Lindberg et al., 2005; Flores-Morales et al., 2006), which

down-regulate GH receptor-JAK pathway by interacting with the receptor-JAK complex (Flores-Morales et al., 2006). In addition, tyrosine phosphatases, such as SHP-1 and -2, are involved in turning off GH signaling (Ram et al., 1997; Gu et al., 2003; Pasquali et al., 2003).

Intracellular pathways in GH signal transduction

Compared with other growth factors, surprisingly little is known about the intracellular events that convey the mitogenic signal of GH into a proliferative response. In addition to the pathways mediated by GH discussed above, activation of GH receptor is shown to undergo ligand-activated translocation into the nucleus through an endosomal route (Davies et al., 2001). Protein kinase A (PKA) (Sirotkin et al., 1999; Yip et al., 1999; Sirotkin et al., 2002; Sirotkin 2005), protein phosphatases (Stofega et al., 1998; Pasquali et al., 2003; Wang et al., 2007) and protein kinase C (PKC) are also shown to be involved in GH actions (Doglio et al., 1989; Smal et al., 1989; Johnson et al., 1990; Sjöholm et al., 2000). In pre-adipocytes, induction of the *c-fos* gene by GH appears to be mediated by PKC. In these cells, GH elicits a rapid accumulation of diacylglycerol (DAG) without a corresponding synthesis of inositol polyphosphates, implying involvement of phospholipid species other than phosphatidylinositol-4,5-bisphosphate (Doglio et al., 1989; Johnson et al., 1990). Our study on rat β -cells showed that GH-stimulated mitogenesis is associated with, in addition to an increase in $[Ca^{2+}]_i$, an increase in DAG content via a phosphatidylcholine-specific PLC, but not MAPKs, PLD, or the cAMP signaling

pathway (Sjoholm et al., 2000). The stimulatory effect of GH on β -cell mitogenesis was curtailed by inhibition of PKC activity or by pretreatment with pertussis toxin, known to influence signal transduction through heterotrimeric GTP-binding proteins. These data indicate an involvement of DAG in translation of the stimulatory signal of GH into a proliferative response in the β -cell, which occurs through GTP-binding proteins and PKC-dependent mechanisms. Later, studies on insulin-secreting INS-1 cells suggest an essential role of JAK2-STAT5 in GH- and PRL-induced β -cell proliferation (Nielsen et al., 2001). The important role of STAT5 in β -cell growth was reinforced in cells expressing a constitutive active STAT5 mutant, which resulted in an enhanced proliferation even in the absence of hGH (Nielsen et al., 2001).

GH regulates Ca^{2+} handling in the pancreatic β -cell

Ca^{2+} activates the β -cell

An increase in the cytoplasmic free Ca^{2+} concentration ($[\text{Ca}^{2+}]_i$) is a primary step in mediating cellular responses in the β -cell, involving proliferation, apoptosis and insulin secretion induced by nutrients, hormones and many other modulators. A key and early element in the highly complex mechanisms of insulin secretion is an increase in $[\text{Ca}^{2+}]_i$. Ca^{2+} influx from the extracellular space, mediated through plasma membrane voltage-gated Ca^{2+} channels, and the subsequent rise in $[\text{Ca}^{2+}]_i$, are crucial for insulin release (Ashcroft 1994; Moosmang et al., 2005). In addition, Ca^{2+} mobilization from intracellular Ca^{2+} stores is an important source of cytosolic Ca^{2+} and plays important role on Ca^{2+} handling in cells. Pancreatic β -cells are equipped

with highly structured Ca^{2+} stores, like the endoplasmic reticulum (ER). There is also evidence for the presence of Ca^{2+} stores in insulin secretory granules (Mitchell et al., 2003). The Ca^{2+} stores feature Ca^{2+} pumps and Ca^{2+} release channels, which are able to modulate depolarization-induced Ca^{2+} signals (da Silva et al., 2000; Sencer et al., 2001). Mobilization of Ca^{2+} from the intracellular stores involves two important Ca^{2+} channels or receptors, viz. inositol 1,4,5-trisphosphate (InsP_3) receptors (InsP_3R) and ryanodine receptors (RyR). The InsP_3R family comprises three members identified to date, named type-1 ($\text{InsP}_3\text{R-1}$), type-2 ($\text{InsP}_3\text{R-2}$) and type-3 InsP_3R ($\text{InsP}_3\text{R-3}$) (Furuichi et al., 1989; Sudhof et al., 1991; Maranto 1994). InsP_3R is a ligand-gated cation channel, capable of mediating Ca^{2+} mobilization in response to InsP_3 , a well-established second messenger involved in Ca^{2+} signaling in many types of cells (Sneyd et al., 2005). Ca^{2+} *per se* is also an important regulator of InsP_3R activity, inasmuch as it can act as a co-agonist to facilitate Ca^{2+} release at low concentrations and inhibit channel activity at high concentrations (Bezprozvanny et al., 1991). Reversible phosphorylation and dephosphorylation of the channel by multifarious kinases and phosphatases also influence channel activity rapidly and substantially (Ammala et al., 1994).

RyRs comprise at least three subtypes, RyR_1 , RyR_2 and RyR_3 . The mRNAs for the three types of RyRs have been cloned and sequenced from mammalian tissues (Sutko et al., 1996). RyR_1 is mainly expressed in muscle cells and in insulin granules of pancreatic β -cells (Mitchell et al., 2001; Mitchell et al., 2003). RyR_2 is abundant in cardiac muscle and it is also the major form of RyR in the β -cell (Lemmens et al.,

2001; Islam 2002). RyR₃ is the main RyR in the brain (Sutko et al., 1996). The RyRs bind the plant alkaloid ryanodine with high affinity. At nanomolar concentrations, ryanodine activates RyRs by increasing the open probability and sensitizing the channel to Ca²⁺, whereas high micromolar concentrations of ryanodine inhibit RyRs (Masumiya et al., 2001). In addition, ryanodine binds to the RyR in the open conformation (Johnson et al., 1990). These divergent actions of ryanodine on the RyRs may create difficulties in its applications under certain experimental conditions. The expression or function of RyR channels in the β -cell is reduced in both diabetic animal models (Islam 2002) and in diabetic patients (Patti et al., 2003), suggesting a role of these channels in the pathogenesis of the disease.

The most important activator of RyR channels is Ca²⁺, which binds to the channel receptor (Meissner 2002). The maximum activity of the RyR is maintained at Ca²⁺ concentrations up to 100 μ M (Bezprozvanny et al., 1991; Meissner 2002), implying that the channel behaves mainly as a Ca²⁺-activated channel under physiological conditions. The involvement of the channel in Ca²⁺-induced Ca²⁺ release (CICR) has been described in different cell types, including the β -cell (Lemmens et al., 2001; Sencer et al., 2001; Islam 2002). CICR is so named because an increase in [Ca²⁺]_i causes further release of Ca²⁺ from intracellular stores by acting on the Ca²⁺-releasing channels, thereby amplifying the Ca²⁺ signal induced by Ca²⁺ entry (Lemmens et al., 2001). Ca²⁺ entry through the voltage-gated L-type Ca²⁺ channel is the main trigger for activation of RyRs. A direct interaction between RyR and the L-type Ca²⁺ channel was also observed in skeletal muscle cells (Sencer et al.,

2001), an example of cross-talk in which the intracellular Ca^{2+} channel activity is directly controlled by the L-type Ca^{2+} channel.

In addition to Ca^{2+} , the gating of the RyR Ca^{2+} channels is modulated by a multitude of factors. It can be activated by adenine nucleotides or caffeine and inhibited by ruthenium red (Islam 2002). RyRs can also be phosphorylated by protein kinases (Suko et al., 1993; MacKrell 1999). Phosphorylation of RyRs enhances the sensitivity of the channels to Ca^{2+} , leading to increased channel opening (Marx et al., 2000). All three mammalian RyR isoforms contain multiple consensus sites for phosphorylation by PKA, which increases the number of cells displaying intracellular Ca^{2+} elevation in response to caffeine (Yoshida et al., 1992). In both human and rat β -cells, glucagon-like peptide-1 (GLP-1) induces Ca^{2+} mobilization by promoting CICR through sensitizing RyRs to Ca^{2+} , an effect that is dependent on cAMP (Holz et al., 1999). The GLP-1 receptor-mediated, cAMP-dependent Ca^{2+} mobilization in the β -cell involves both PKA and cAMP-regulated guanine nucleotide exchange factor (cAMP-GEF-II, Epac2) (Kang et al., 2001; Kang et al., 2005). In addition to serine/threonine kinases, tyrosine kinases also phosphorylate RyRs and are involved in RyR-mediated Ca^{2+} mobilization (Guse et al., 2001). There are indications of involvement of CICR in the exocytosis of insulin (Kang et al., 2003; Dyachok et al., 2004), suggesting that exocytosis in β -cells may not be simply dependent on Ca^{2+} influx through the voltage-dependent Ca^{2+} channels, but also dependent on the interaction of Ca^{2+} to promote CICR.

hGH-induced rise in $[Ca^{2+}]_i$ is dependent on extracellular Ca^{2+} , membrane potential and Ca^{2+} influx through the voltage-gated L-type Ca^{2+} -channel in rat insulin-secreting β -cells

A rise in $[Ca^{2+}]_i$ has been observed in different cellular events induced by GH, such as gene transcription (Billestrup et al., 1995; Sjöholm et al., 2000), cell growth (Sjöholm et al., 2000; Nielsen et al., 2001), carbohydrate metabolism (Schwartz et al., 1992) and hormone secretion (Sekine et al., 1996). Previous studies on Ca^{2+} handling in different tissues showed that both Ca^{2+} influx and Ca^{2+} mobilization are involved in GH-stimulated increase in $[Ca^{2+}]_i$ (Billestrup et al., 1995; Gaur et al., 1996; Sekine et al., 1996; Boquet et al., 1997). The molecular mechanisms underlying GH-regulated Ca^{2+} handling have not been fully understood. In rat β -cells, administration of hGH caused a rapid increase in $[Ca^{2+}]_i$ (Sjöholm et al., 2000; Zhang et al., 2004; Zhang et al., 2006). Further studies in the rat insulin-secreting BRIN-BD11 cell line showed that the effect of hGH was dependent on extracellular Ca^{2+} , as hGH failed to induce any effect on $[Ca^{2+}]_i$ in the absence of ambient Ca^{2+} . The manner of the hGH action indicates a requirement of Ca^{2+} influx in hGH-induced rise in $[Ca^{2+}]_i$. In addition, the effect of hGH was abolished in the presence of the K_{ATP} channel opener diazoxide, suggesting an important role of membrane potential in the GH action (Zhang et al., 2004). In β -cells, opening of voltage-gated L-type Ca^{2+} channels by depolarization of the plasma membrane is the quantitatively most important mechanism in raising $[Ca^{2+}]_i$. The hGH-induced rise in $[Ca^{2+}]_i$ was completely nullified by the L-type Ca^{2+}

channel blocker nifedipine, thus lending support to an involvement of the L-type Ca^{2+} channel in raising $[\text{Ca}^{2+}]_i$. However, hGH induced changes neither in Ca^{2+} current, as demonstrated by the patch-clamp technique, nor in membrane potential, as examined by the patch-clamp technique or by using potential-sensitive dyes. These results imply that hGH *per se* does not directly interfere with the activity of the channels and membrane potential, rather a certain amount of Ca^{2+} getting into the cells through the L-type Ca^{2+} channel seems to be required for the action of hGH.

The permissive role of Ca^{2+} entry through the L-type Ca^{2+} channel for the GH action is further evident from studies in the absence of glucose (Fig. 1). hGH fails to influence $[\text{Ca}^{2+}]_i$ at 0 mM glucose (Fig. 1B), while a robust response occurs in the presence of 3 mM of the sugar. Actually, Ca^{2+} entry through the L-type Ca^{2+} channel occurs already at non-stimulatory concentration of glucose (Navedo et al., 2005). Indeed, additional experiments revealed that a slight increase in $[\text{Ca}^{2+}]_i$ is already induced by 3 mM glucose (Fig. 1A). This indicates that, while hGH does not induce Ca^{2+} entry, certain Ca^{2+} influx through the L-type channel is a prerequisite for hGH-stimulated rise in $[\text{Ca}^{2+}]_i$.

Fig 1. Effect of hGH on $[Ca^{2+}]_i$ in the presence or absence of glucose. Rat insulin-secreting BRIN-BD11 cells on cover slips were loaded with Fura-2 and perfused with buffer A containing 0 or 3 mM glucose. $[Ca^{2+}]_i$ measurement was performed on cell clusters (3-5 cells). Addition of hGH (GH, 25 nM) is indicated by horizontal bars. Cells were depolarized by 25 mM K^+ at the end of the experiments as a positive control.

hGH raises $[Ca^{2+}]_i$ by facilitating CICR through tyrosine phosphorylation of RyR in the rat insulin-secreting β -cell

The findings in the rat insulin-secreting β -cell that hGH does not interfere with Ca^{2+} entry, but requires Ca^{2+} entry through the voltage-gated L-type Ca^{2+} channel, suggest an involvement of CICR in the action of hGH. Several pieces of evidence implicate a crucial role of intracellular Ca^{2+} pools in hGH-induced rise in $[Ca^{2+}]_i$ in our studies (Zhang et al., 2004). First, depletion of the intracellular Ca^{2+} pools by the ER Ca^{2+} -ATPase inhibitor thapsigargin blocked the effect of hGH. Second, in the presence of ruthenium red, known to inhibit mitochondrial Ca^{2+} transport and ER Ca^{2+} release channels, hGH-induced rise in $[Ca^{2+}]_i$ was completely abolished. Third, hGH was unable to raise $[Ca^{2+}]_i$ after pretreatment of the cells with caffeine. Finally, stimulation of rat β -cells with hGH evoked an increased synthesis of diacylglycerol, but not $InsP_3$ production (Sjoholm et al., 2000), the latter being required for $InsP_3R$ activation. In their entirety, these findings point to a role of RyRs in the action of GH, but the type of RyR involved remains unknown. Indeed, stimulation of the cells with hGH caused rapid tyrosine phosphorylation of RyRs. A marked phosphorylation of

RyRs was observed after 2 min of stimulation and declined thereafter (Zhang et al., 2004). This activation pattern of RyRs is consistent with tyrosine phosphorylation of the receptors as reported in T cells (Guse et al., 2001). When tyrosine kinase activity was inhibited, the effect of hGH on $[Ca^{2+}]_i$ was completely abolished, indicating a crucial role of tyrosine phosphorylation of RyRs in hGH-induced CICR. CICR does not occur without hGH stimulation in the presence of 3 mM glucose, indicating that hGH-induced tyrosine phosphorylation of RyRs sensitizes the channel to Ca^{2+} , getting into the cells through the L-type Ca^{2+} channel. The hGH-induced sensitization in the CICR process was further evidenced in K^+ -induced rise in $[Ca^{2+}]_i$, which was significantly enhanced after pre-treatment of the cells with hGH. Hence, hGH-stimulated rise in $[Ca^{2+}]_i$ is mediated by CICR through tyrosine phosphorylation of RyRs.

Tyrosine phosphorylation mediated by JAK2 and Src tyrosine kinases play a crucial role in hGH-induced $[Ca^{2+}]_i$ and insulin secretion

The requirement for tyrosine phosphorylation of RyRs in the effects of hGH immediately raised an interesting question: Which tyrosine kinase is involved in the hGH action? Previous studies on Chinese hamster ovary (CHO) cells transfected with GHR or PRLR were shown that activation of JAK2 and tyrosine phosphorylation are required for PRLR-, but not GHR-mediated rise in $[Ca^{2+}]_i$, while is required for rat GH-induced gene transcription (Billestrup et al., 1995; Prevarskaya et al., 1995; Sorin et al., 2000). This indicates that a rise in $[Ca^{2+}]_i$ is not directly involved in rat

GH-induced proliferation in the β -cell and that the regulation of Ca^{2+} handling by the two close, but distinct receptors may differ.

Stimulation of the rat β -cells with hGH causes rapid tyrosyl phosphorylation of both JAK2 and c-Src, which is abolished by inhibitors of these kinases (Zhang et al., 2006). Application of two different inhibitors for either JAK2 or Src kinases also blocks the hGH-induced rise in $[\text{Ca}^{2+}]_i$, without significant effects on depolarization-induced rise in $[\text{Ca}^{2+}]_i$, suggesting a requirement of JAK2 and Src kinases for the GH actions. In addition, the inhibitors did not affect the ability of bovine GH (bGH) to raise $[\text{Ca}^{2+}]_i$. The notion that the specific tyrosine kinase inhibitors do not influence Ca^{2+} influx through the L-type Ca^{2+} channels, while effectively abolishing the hGH effect, adds further credence to the view that Ca^{2+} influx *per se* is not directly involved in the effect of hGH on $[\text{Ca}^{2+}]_i$ in the insulin-secreting cells. The complete inhibition of the effects evoked by hGH by specifically inhibiting either JAK2 or Src kinases suggests a downstream activation of Src after JAK2 activation. Activation of JAK2 kinase has been shown to be associated with Src tyrosine kinases, especially c-Src and fyn (Berlanga et al., 1995). Considering that a ligand-dependent direct binding of JAK2 with Src kinases was reported in vascular smooth muscle cells stimulated with angiotensin II (Sandberg et al., 2005), such a physical interaction would allow JAK2 to act through Src kinases.

hGH-induced rise in $[\text{Ca}^{2+}]_i$ and insulin secretion in rat insulin-secreting β -cells are mainly conveyed through the PRL receptor

Although PRL and GH receptors share many similarities in structure and function, signaling through the different receptors differs in several aspects (Brelje et al., 1991; Billestrup et al., 1995; Prevarskaya et al., 1995; Sorin et al., 2000). Since hGH binds and activates both GH and PRL receptors in human and rodents, the receptor type transducing the actions of hGH was scrutinized. To this end, bGH, which exclusively binds to the GH receptor, and ovine PRL (oPRL), which interacts solely with the PRL receptor, were applied in the studies (Zhang et al., 2006). Both oPRL and bGH raised $[Ca^{2+}]_i$ in the rat insulin-secreting β -cell line BRIN-BD11. However, hGH- or oPRL-, but not bGH-induced rise in $[Ca^{2+}]_i$ was sensitive to the tyrosine kinase inhibitors and was associated with enhanced insulin release. Although hGH is able to interact with both rat GH and PRL receptors, the above results indicate a requirement of tyrosine phosphorylation for PRL receptor-, but not GH receptor-mediated Ca^{2+} signaling in these cells, thus favoring the idea of an involvement of PRL receptor, rather than GH receptor occupancy, in the hGH-induced events. Such a scenario is consistent with the previous findings that hGH functions mainly through the PRL receptor in rodent islets (Brelje et al., 1991) and that signaling through the PRL receptor is considerably more effective than the GH receptor in the enhancement of islet function *in vitro* (Brelje et al., 1991; Weinhaus et al., 1996; Nielsen et al., 2001; Freemark et al., 2002).

Figure 2. Schematic model of hGH-induced rise in $[Ca^{2+}]_i$ in the rat β -cell. In the presence of non-stimulatory glucose concentrations, a certain amount of Ca^{2+} enters the cells through the voltage-gated L-type Ca^{2+} channel without triggering CICR (A). hGH or PRL, through interaction with PRLR, activates the tyrosine kinases JAK2 and Src, resulting in tyrosine phosphorylation of RyRs (R). The latter event sensitizes RyRs to Ca^{2+} getting into the cells through the L-type Ca^{2+} channel, thereby activating CICR. Increase in $[Ca^{2+}]_i$ promotes insulin secretion (B).

Tyrosine phosphorylation plays a role in initiation of CICR and insulin secretion induced by hGH

In spite of the occurrence of CICR in pancreatic β -cells, corroborated by many studies (Johnson et al., 1990; Graves et al., 2003; Mitchell et al., 2003; Kang et al., 2005), it is unclear to what extent CICR participates in the regulation of insulin secretion. Stimulation of human β -cells with nanomolar concentrations of ryanodine evoked increases in $[Ca^{2+}]_i$, associated with a transient release of insulin (Johnson et al., 1990). The ryanodine-induced insulin secretion was abolished by an intracellular Ca^{2+} chelator, indicating a direct linkage between insulin secretion and cytosolic Ca^{2+}

and an involvement of RyRs in insulin secretion. Our study in the rat insulin-secreting β -cell line BRIN-BD11 demonstrated that hGH-induced rise in $[Ca^{2+}]_i$ through activation of CICR is associated with, and required for, an increased insulin secretion (Zhang et al., 2006). In contrast, bGH failed to stimulate insulin secretion despite an increase in $[Ca^{2+}]_i$. The absence of a secretory response to bGH suggests that an increase in $[Ca^{2+}]_i$ is not sufficient for stimulating secretion under these circumstances. This notion was further strengthened by the observation that PP2 completely inhibited hGH-induced insulin secretion from the β -cells, while only partially inhibiting the Ca^{2+} response to the hormone. The above observations collectively led to the conclusion that a rise in $[Ca^{2+}]_i$ elicited by activation of PRLR is JAK2-dependent and is associated with enhanced insulin secretion. In contrast, GH receptor-mediated increase in $[Ca^{2+}]_i$ is JAK2-independent and is dissociated from insulin secretion. Since hGH raises $[Ca^{2+}]_i$ through CICR via the PRL receptor, and blocking the $[Ca^{2+}]_i$ rise by tyrosine kinase inhibitors abolishes the hormone-stimulated insulin secretion, it can be deduced that CICR directly participates in insulin secretion stimulated by hGH or PRL in insulin-secreting cells.

Concluding remarks and future perspectives

Identification of the intracellular signals initiated by GH provides remarkable insights into the multiple pathways that control function and growth of the pancreatic β -cell. Nevertheless, many molecular and cellular aspects of the flow of information transduced by GH remain unclear. The genetic bases and the molecular steps by

which GH- or PRL-receptor occupancy is selected and coupled to the generation of the early regulatory signals remain important areas for future research. Target-mutation or knock-down of the RyR in insulin-secreting cells will be a useful model to characterize the direct role of RyR and CICR on Ca^{2+} handling and insulin secretion mediated through the PRL receptor. In addition, studies on GH-PRL signaling in purified human pancreatic β -cells from healthy and type 2 diabetic donors (Parnaud et al., 2008) would provide direct evidence of the functions of these receptors. Such information can be harnessed to advantage with regard to a potential application of the lactogen-induced CICR and insulin secretion in pharmaceutical development and management of diabetes, especially gestational diabetes. Considering that Ca^{2+} handling regulated by the GH receptor differs from the PRL receptor, and that rise in $[\text{Ca}^{2+}]_i$ is thought to be an early event in GH-mediated metabolic or mitogenic actions (Rozengurt 1986; Billestrup et al., 1995; Sjöholm et al., 2000), clarification of Ca^{2+} handling and the signal transduction pathway mediated through the GH receptor will further illuminate the roles of these two close but distinct receptors in pancreatic β -cell biology.

References

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- Abdul-Ghani, M. A., Tripathy, D. and DeFronzo, R. A. (2006). Contributions of beta-cell dysfunction and insulin resistance to the pathogenesis of impaired glucose tolerance and impaired fasting glucose. *Diabetes Care* 29(5): 1130-9.
- al-Sakkaf, K. A., Dobson, P. R. and Brown, B. L. (1997). Prolactin induced tyrosine phosphorylation of p59fyn may mediate phosphatidylinositol 3-kinase activation in Nb2 cells. *J Mol Endocrinol* 19(3): 347-50.

- Al-Sakkaf, K. A., Mooney, L. M., Dobson, P. R. and Brown, B. L. (2000). Possible role for protein kinase B in the anti-apoptotic effect of prolactin in rat Nb2 lymphoma cells. *J Endocrinol* 167(1): 85-92.
- Ammala, C., Eliasson, L., Bokvist, K., Berggren, P. O., Honkanen, R. E., Sjöholm, A. and Rorsman, P. (1994). Activation of protein kinases and inhibition of protein phosphatases play a central role in the regulation of exocytosis in mouse pancreatic beta cells. *Proc Natl Acad Sci U S A* 91(10): 4343-7.
- Argetsinger, L. S., Campbell, G. S., Yang, X., Witthuhn, B. A., Silvennoinen, O., Ihle, J. N. and Carter-Su, C. (1993). Identification of JAK2 as a growth hormone receptor-associated tyrosine kinase. *Cell* 74(2): 237-44.
- Ashcroft, S. J. (1994). Protein phosphorylation and beta-cell function. *Diabetologia* 37 Suppl 2: S21-9.
- Banhegyi, G., Baumeister, P., Benedetti, A., Dong, D., Fu, Y., Lee, A. S., Li, J., Mao, C., Margittai, E., Ni, M., Paschen, W., Piccirella, S., Senesi, S., Sitia, R., Wang, M. and Yang, W. (2007). Endoplasmic reticulum stress. *Ann N Y Acad Sci* 1113: 58-71.
- Bell, G. I. and Polonsky, K. S. (2001). Diabetes mellitus and genetically programmed defects in beta-cell function. *Nature* 414(6865): 788-91.
- Berlanga, J. J., Fresno Vara, J. A., Martin-Perez, J. and Garcia-Ruiz, J. P. (1995). Prolactin receptor is associated with c-src kinase in rat liver. *Mol Endocrinol* 9(11): 1461-7.
- Bezprozvanny, I., Watras, J. and Ehrlich, B. E. (1991). Bell-shaped calcium-response curves of Ins(1,4,5)P₃- and calcium-gated channels from endoplasmic reticulum of cerebellum. *Nature* 351(6329): 751-4.
- Billestrup, N., Bouchelouche, P., Allevato, G., Ilondo, M. and Nielsen, J. H. (1995). Growth hormone receptor C-terminal domains required for growth hormone-induced intracellular free Ca²⁺ oscillations and gene transcription. *Proc Natl Acad Sci U S A* 92(7): 2725-9.
- Billestrup, N. and Nielsen, J. H. (1991). The stimulatory effect of growth hormone, prolactin, and placental lactogen on beta-cell proliferation is not mediated by insulin-like growth factor-I. *Endocrinology* 129(2): 883-8.
- Bjorge, J. D., Jakymiw, A. and Fujita, D. J. (2000). Selected glimpses into the activation and function of Src kinase. *Oncogene* 19(49): 5620-35.
- Bonner-Weir, S. (2000). Life and death of the pancreatic beta cells. *Trends Endocrinol Metab* 11(9): 375-8.
- Bonner-Weir, S. (2000). Perspective: Postnatal pancreatic beta cell growth. *Endocrinology* 141(6): 1926-9.
- Boquet, G., Barakat, L., Paly, J., Djiane, J. and Dufy, B. (1997). Involvement of both calcium influx and calcium mobilization in growth hormone-induced [Ca²⁺]_i increases in Chinese hamster ovary cells. *Mol Cell Endocrinol* 131(1): 109-20.
- Brelje, T. C. and Sorenson, R. L. (1991). Role of prolactin versus growth hormone on islet B-cell proliferation in vitro: implications for pregnancy. *Endocrinology* 128(1): 45-57.
- Carter-Su, C., Argetsinger, L. S., Campbell, G. S., Wang, X., Ihle, J. and Witthuhn, B. (1994). The identification of JAK2 tyrosine kinase as a signaling molecule for growth hormone. *Proc Soc Exp Biol Med* 206(3): 210-5.
- Clevenger, C. V. and Medaglia, M. V. (1994). The protein tyrosine kinase P59fyn is associated with prolactin (PRL) receptor and is activated by PRL stimulation of T-lymphocytes. *Mol Endocrinol* 8(6): 674-81.

- da Silva, C. P. and Guse, A. H. (2000). Intracellular Ca^{2+} release mechanisms: multiple pathways having multiple functions within the same cell type? *Biochim Biophys Acta* 1498(2-3): 122-33.
- Davies, G. F., Khandelwal, R. L., Wu, L., Juurlink, B. H. and Roesler, W. J. (2001). Inhibition of phosphoenolpyruvate carboxykinase (PEPCK) gene expression by troglitazone: a peroxisome proliferator-activated receptor- γ (PPAR γ)-independent, antioxidant-related mechanism. *Biochem Pharmacol* 62(8): 1071-9.
- Dickson, L. M. and Rhodes, C. J. (2004). Pancreatic beta-cell growth and survival in the onset of type 2 diabetes: a role for protein kinase B in the Akt? *Am J Physiol Endocrinol Metab* 287(2): E192-8.
- Doglio, A., Dani, C., Grimaldi, P. and Ailhaud, G. (1989). Growth hormone stimulates c-fos gene expression by means of protein kinase C without increasing inositol lipid turnover. *Proc Natl Acad Sci U S A* 86(4): 1148-52.
- Dominici, F. P., Argentino, D. P., Munoz, M. C., Miquet, J. G., Sotelo, A. I. and Turyn, D. (2005). Influence of the crosstalk between growth hormone and insulin signalling on the modulation of insulin sensitivity. *Growth Horm IGF Res* 15(5): 324-36.
- Dyachok, O. and Gylfe, E. (2004). Ca^{2+} -induced Ca^{2+} release via inositol 1,4,5-trisphosphate receptors is amplified by protein kinase A and triggers exocytosis in pancreatic beta-cells. *J Biol Chem* 279(44): 45455-61.
- Feng, J., Witthuhn, B. A., Matsuda, T., Kohlhuber, F., Kerr, I. M. and Ihle, J. N. (1997). Activation of Jak2 catalytic activity requires phosphorylation of Y1007 in the kinase activation loop. *Mol Cell Biol* 17(5): 2497-501.
- Fernandez, A. M., Kim, J. K., Yakar, S., Dupont, J., Hernandez-Sanchez, C., Castle, A. L., Filmore, J., Shulman, G. I. and Le Roith, D. (2001). Functional inactivation of the IGF-I and insulin receptors in skeletal muscle causes type 2 diabetes. *Genes Dev* 15(15): 1926-34.
- Flores-Morales, A., Greenhalgh, C. J., Norstedt, G. and Rico-Bautista, E. (2006). Negative regulation of growth hormone receptor signaling. *Mol Endocrinol* 20(2): 241-53.
- Foster, C. M., Shafer, J. A., Rozsa, F. W., Wang, X. Y., Lewis, S. D., Renken, D. A., Natale, J. E., Schwartz, J. and Carter-Su, C. (1988). Growth hormone promoted tyrosyl phosphorylation of growth hormone receptors in murine 3T3-F442A fibroblasts and adipocytes. *Biochemistry* 27(1): 326-34.
- Freemark, M., Avril, I., Fleenor, D., Driscoll, P., Petro, A., Opara, E., Kendall, W., Oden, J., Bridges, S., Binart, N., Breant, B. and Kelly, P. A. (2002). Targeted deletion of the PRL receptor: effects on islet development, insulin production, and glucose tolerance. *Endocrinology* 143(4): 1378-85.
- Furuichi, T., Yoshikawa, S., Miyawaki, A., Wada, K., Maeda, N. and Mikoshiba, K. (1989). Primary structure and functional expression of the inositol 1,4,5-trisphosphate-binding protein P400. *Nature* 342(6245): 32-8.
- Garay, G. L., Akerblom, H. K. and Martin, J. M. (1971). Experimental hypersomatotropism: serum growth hormone and insulin, and pituitary and pancreatic changes in MtT-W15 tumor-bearing rats before and after tumor removal. *Horm Metab Res* 3(2): 82-9.
- Gaur, S., Yamaguchi, H. and Goodman, H. M. (1996). Growth hormone regulates cytosolic free calcium in rat fat cells by maintaining L-type calcium channels. *Am J Physiol* 270(5 Pt 1): C1478-84.

- Gloyn, A. L., Weedon, M. N., Owen, K. R., Turner, M. J., Knight, B. A., Hitman, G., Walker, M., Levy, J. C., Sampson, M., Halford, S., McCarthy, M. I., Hattersley, A. T. and Frayling, T. M. (2003). Large-scale association studies of variants in genes encoding the pancreatic beta-cell KATP channel subunits Kir6.2 (KCNJ11) and SUR1 (ABCC8) confirm that the KCNJ11 E23K variant is associated with type 2 diabetes. *Diabetes* 52(2): 568-72.
- Goodarzi, M. O., Lehman, D. M., Taylor, K. D., Guo, X., Cui, J., Quinones, M. J., Clee, S. M., Yandell, B. S., Blangero, J., Hsueh, W. A., Attie, A. D., Stern, M. P. and Rotter, J. I. (2007). SORCS1: a novel human type 2 diabetes susceptibility gene suggested by the mouse. *Diabetes* 56(7): 1922-9.
- Graves, T. K. and Hinkle, P. M. (2003). Ca(2+)-induced Ca(2+) release in the pancreatic beta-cell: direct evidence of endoplasmic reticulum Ca(2+) release. *Endocrinology* 144(8): 3565-74.
- Gu, F., Dube, N., Kim, J. W., Cheng, A., Ibarra-Sanchez Mde, J., Tremblay, M. L. and Boisclair, Y. R. (2003). Protein tyrosine phosphatase 1B attenuates growth hormone-mediated JAK2-STAT signaling. *Mol Cell Biol* 23(11): 3753-62.
- Guo, Y., Lu, Y., Houle, D., Robertson, K., Tang, Z., Kopchick, J. J., Liu, Y. L. and Liu, J. L. (2005). Pancreatic islet-specific expression of an insulin-like growth factor-I transgene compensates islet cell growth in growth hormone receptor gene-deficient mice. *Endocrinology* 146(6): 2602-9.
- Guse, A. H., Tsygankov, A. Y., Weber, K. and Mayr, G. W. (2001). Transient tyrosine phosphorylation of human ryanodine receptor upon T cell stimulation. *J Biol Chem* 276(37): 34722-7.
- Hellerstrom, C., Sjöholm, A. and Swenne, I. (1991). Effects of growth hormone and related growth factors on DNA replication and insulin production in pancreatic islet beta-cells. *Acta Paediatr Scand Suppl* 377: 55-62; discussion 63.
- Hellman, B. and Angervall, L. (1961). The frequency distribution of the number and volume of the islets of Langerhans in man. 3. Studies in diabetes of early onset, insuloma and acromegaly. *Acta Pathol Microbiol Scand* 53: 230-6.
- Holz, G. G., Leech, C. A., Heller, R. S., Castonguay, M. and Habener, J. F. (1999). cAMP-dependent mobilization of intracellular Ca²⁺ stores by activation of ryanodine receptors in pancreatic beta-cells. A Ca²⁺ signaling system stimulated by the insulinotropic hormone glucagon-like peptide-1-(7-37). *J Biol Chem* 274(20): 14147-56.
- Islam, M. S. (2002). The ryanodine receptor calcium channel of beta-cells: molecular regulation and physiological significance. *Diabetes* 51(5): 1299-309.
- Johnson, R. M., Napier, M. A., Cronin, M. J. and King, K. L. (1990). Growth hormone stimulates the formation of sn-1,2-diacylglycerol in rat hepatocytes. *Endocrinology* 127(5): 2099-103.
- Johnston, J. A., Kawamura, M., Kirken, R. A., Chen, Y. Q., Blake, T. B., Shibuya, K., Ortaldo, J. R., McVicar, D. W. and O'Shea, J. J. (1994). Phosphorylation and activation of the Jak-3 Janus kinase in response to interleukin-2. *Nature* 370(6485): 151-3.
- Kahn, S. E. and Halban, P. A. (1997). Release of incompletely processed proinsulin is the cause of the disproportionate proinsulinemia of NIDDM. *Diabetes* 46(11): 1725-32.
- Kahn, S. E., Leonetti, D. L., Prigeon, R. L., Boyko, E. J., Bergstrom, R. W. and Fujimoto, W. Y. (1995). Relationship of proinsulin and insulin with noninsulin-dependent diabetes mellitus and coronary heart disease in Japanese-American men: impact of obesity--clinical research center study. *J Clin Endocrinol Metab* 80(4): 1399-406.

- Kang, G., Chepurny, O. G. and Holz, G. G. (2001). cAMP-regulated guanine nucleotide exchange factor II (Epac2) mediates Ca^{2+} -induced Ca^{2+} release in INS-1 pancreatic beta-cells. *J Physiol* 536(Pt 2): 375-85.
- Kang, G., Chepurny, O. G., Rindler, M. J., Collis, L., Chepurny, Z., Li, W. H., Harbeck, M., Roe, M. W. and Holz, G. G. (2005). A cAMP and Ca^{2+} coincidence detector in support of Ca^{2+} -induced Ca^{2+} release in mouse pancreatic beta cells. *J Physiol* 566(Pt 1): 173-88.
- Kang, G. and Holz, G. G. (2003). Amplification of exocytosis by Ca^{2+} -induced Ca^{2+} release in INS-1 pancreatic beta cells. *J Physiol* 546(Pt 1): 175-89.
- Lemmens, R., Larsson, O., Berggren, P. O. and Islam, M. S. (2001). Ca^{2+} -induced Ca^{2+} release from the endoplasmic reticulum amplifies the Ca^{2+} signal mediated by activation of voltage-gated L-type Ca^{2+} channels in pancreatic beta-cells. *J Biol Chem* 276(13): 9971-7.
- Lindberg, K., Ronn, S. G., Tornehave, D., Richter, H., Hansen, J. A., Romer, J., Jackerott, M. and Billestrup, N. (2005). Regulation of pancreatic beta-cell mass and proliferation by SOCS-3. *J Mol Endocrinol* 35(2): 231-43.
- Lingohr, M. K., Buettner, R. and Rhodes, C. J. (2002). Pancreatic beta-cell growth and survival--a role in obesity-linked type 2 diabetes? *Trends Mol Med* 8(8): 375-84.
- Lis, J. and Wu, C. (1993). Protein traffic on the heat shock promoter: parking, stalling, and trucking along. *Cell* 74(1): 1-4.
- Liu, J. L., Coschigano, K. T., Robertson, K., Lipsett, M., Guo, Y., Kopchick, J. J., Kumar, U. and Liu, Y. L. (2004). Disruption of growth hormone receptor gene causes diminished pancreatic islet size and increased insulin sensitivity in mice. *Am J Physiol Endocrinol Metab* 287(3): E405-13.
- Loos, R. J., Franks, P. W., Francis, R. W., Barroso, I., Gribble, F. M., Savage, D. B., Ong, K. K., O'Rahilly, S. and Wareham, N. J. (2007). TCF7L2 polymorphisms modulate proinsulin levels and beta-cell function in a British European population. *Diabetes* 56(7): 1943-7.
- Lu, Y., Herrera, P. L., Guo, Y., Sun, D., Tang, Z., LeRoith, D. and Liu, J. L. (2004). Pancreatic-specific inactivation of IGF-I gene causes enlarged pancreatic islets and significant resistance to diabetes. *Diabetes* 53(12): 3131-41.
- MacKrell, J. J. (1999). Protein-protein interactions in intracellular Ca^{2+} -release channel function. *Biochem J* 337 (Pt 3): 345-61.
- Maranto, A. R. (1994). Primary structure, ligand binding, and localization of the human type 3 inositol 1,4,5-trisphosphate receptor expressed in intestinal epithelium. *J Biol Chem* 269(2): 1222-30.
- Marx, S. O., Reiken, S., Hisamatsu, Y., Jayaraman, T., Burkhoff, D., Rosembly, N. and Marks, A. R. (2000). PKA phosphorylation dissociates FKBP12.6 from the calcium release channel (ryanodine receptor): defective regulation in failing hearts. *Cell* 101(4): 365-76.
- Masumiya, H., Li, P., Zhang, L. and Chen, S. R. (2001). Ryanodine sensitizes the Ca^{2+} release channel (ryanodine receptor) to Ca^{2+} activation. *J Biol Chem* 276(43): 39727-35.
- Meissner, G. (2002). Regulation of mammalian ryanodine receptors. *Front Biosci* 7: d2072-80.
- Mitchell, K. J., Lai, F. A. and Rutter, G. A. (2003). Ryanodine receptor type I and nicotinic acid adenine dinucleotide phosphate receptors mediate Ca^{2+} release from insulin-containing vesicles in living pancreatic beta-cells (MIN6). *J Biol Chem* 278(13): 11057-64.
- Mitchell, K. J., Pinton, P., Varadi, A., Tacchetti, C., Ainscow, E. K., Pozzan, T., Rizzuto, R. and Rutter, G. A. (2001). Dense core secretory vesicles revealed as a dynamic Ca^{2+} store in

- neuroendocrine cells with a vesicle-associated membrane protein aequorin chimera. *J Cell Biol* 155(1): 41-51.
- Mizuno, A., Arai, H., Fukaya, M., Sato, M., Hisami, Y. O., Takeda, E. and Doi, T. (2007). Early-phase insulin secretion is disturbed in obese subjects with glucose intolerance. *Metabolism* 56(6): 856-62.
- Moldrup, A., Allevato, G., Dyrberg, T., Nielsen, J. H. and Billestrup, N. (1991). Growth hormone action in rat insulinoma cells expressing truncated growth hormone receptors. *J Biol Chem* 266(26): 17441-5.
- Moldrup, A., Billestrup, N. and Nielsen, J. H. (1990). Rat insulinoma cells express both a 115-kDa growth hormone receptor and a 95-kDa prolactin receptor structurally related to the hepatic receptors. *J Biol Chem* 265(15): 8686-90.
- Moosmang, S., Lenhardt, P., Haider, N., Hofmann, F. and Wegener, J. W. (2005). Mouse models to study L-type calcium channel function. *Pharmacol Ther* 106(3): 347-55.
- Moutoussamy, S., Kelly, P. A. and Finidori, J. (1998). Growth-hormone-receptor and cytokine-receptor-family signaling. *Eur J Biochem* 255(1): 1-11.
- Navedo, M. F., Amberg, G. C., Votaw, V. S. and Santana, L. F. (2005). Constitutively active L-type Ca²⁺ channels. *Proc Natl Acad Sci U S A* 102(31): 11112-7.
- Neubauer, H., Cumano, A., Muller, M., Wu, H., Huffstadt, U. and Pfeffer, K. (1998). Jak2 deficiency defines an essential developmental checkpoint in definitive hematopoiesis. *Cell* 93(3): 397-409.
- Nielsen, J. H., Galsgaard, E. D., Moldrup, A., Friedrichsen, B. N., Billestrup, N., Hansen, J. A., Lee, Y. C. and Carlsson, C. (2001). Regulation of beta-cell mass by hormones and growth factors. *Diabetes* 50 Suppl 1: S25-9.
- Nielsen, J. H., Linde, S., Welinder, B. S., Billestrup, N. and Madsen, O. D. (1989). Growth hormone is a growth factor for the differentiated pancreatic beta-cell. *Mol Endocrinol* 3(1): 165-73.
- Nielsen, J. H., Moldrup, A. and Billestrup, N. (1990). Expression of the growth hormone receptor gene in insulin producing cells. *Biomed Biochim Acta* 49(12): 1151-5.
- Okuda, Y., Pena, J., Chou, J. and Field, J. B. (2001). Acute effects of growth hormone on metabolism of pancreatic hormones, glucose and ketone bodies. *Diabetes Res Clin Pract* 53(1): 1-8.
- Parganas, E., Wang, D., Stravopodis, D., Topham, D. J., Marine, J. C., Teglund, S., Vanin, E. F., Bodner, S., Colamonici, O. R., van Deursen, J. M., Grosveld, G. and Ihle, J. N. (1998). Jak2 is essential for signaling through a variety of cytokine receptors. *Cell* 93(3): 385-95.
- Parnaud, G., Bosco, D., Berney, T., Pattou, F., Kerr-Conte, J., Donath, M. Y., Bruun, C., Mandrup-Poulsen, T., Billestrup, N. and Halban, P. A. (2008). Proliferation of sorted human and rat beta cells. *Diabetologia* 51(1): 91-100.
- Parsons, J. A., Bartke, A. and Sorenson, R. L. (1995). Number and size of islets of Langerhans in pregnant, human growth hormone-expressing transgenic, and pituitary dwarf mice: effect of lactogenic hormones. *Endocrinology* 136(5): 2013-21.
- Parsons, J. A., Hartfel, M. A., Hegre, O. D. and McEvoy, R. C. (1983). Effect of MfTW15 mammosomatotropic tumors on pancreatic islet hormones. *Diabetes* 32(1): 67-74.
- Pasquali, C., Curchod, M. L., Walchli, S., Espanel, X., Guerrier, M., Arigoni, F., Strous, G. and Van Huijsduijnen, R. H. (2003). Identification of protein tyrosine phosphatases with specificity for the ligand-activated growth hormone receptor. *Mol Endocrinol* 17(11): 2228-39.

- Patti, M. E., Butte, A. J., Crunkhorn, S., Cusi, K., Berria, R., Kashyap, S., Miyazaki, Y., Kohane, I., Costello, M., Saccone, R., Landaker, E. J., Goldfine, A. B., Mun, E., DeFronzo, R., Finlayson, J., Kahn, C. R. and Mandarino, L. J. (2003). Coordinated reduction of genes of oxidative metabolism in humans with insulin resistance and diabetes: Potential role of PGC1 and NRF1. *Proc Natl Acad Sci U S A* 100(14): 8466-71.
- Pick, A., Clark, J., Kubstrup, C., Levisetti, M., Pugh, W., Bonner-Weir, S. and Polonsky, K. S. (1998). Role of apoptosis in failure of beta-cell mass compensation for insulin resistance and beta-cell defects in the male Zucker diabetic fatty rat. *Diabetes* 47(3): 358-64.
- Porksen, N. (2002). The in vivo regulation of pulsatile insulin secretion. *Diabetologia* 45(1): 3-20.
- Prevarskaya, N. B., Skryma, R. N., Vacher, P., Daniel, N., Djiane, J. and Dufy, B. (1995). Role of tyrosine phosphorylation in potassium channel activation. Functional association with prolactin receptor and JAK2 tyrosine kinase. *J Biol Chem* 270(41): 24292-9.
- Ram, P. A. and Waxman, D. J. (1997). Interaction of growth hormone-activated STATs with SH2-containing phosphotyrosine phosphatase SHP-1 and nuclear JAK2 tyrosine kinase. *J Biol Chem* 272(28): 17694-702.
- Rhodes, C. J. (2000). IGF-I and GH post-receptor signaling mechanisms for pancreatic beta-cell replication. *J Mol Endocrinol* 24(3): 303-11.
- Rozengurt, E. (1986). Early signals in the mitogenic response. *Science* 234(4773): 161-6.
- Sandberg, E. M., Ma, X., He, K., Frank, S. J., Ostrov, D. A. and Sayeski, P. P. (2005). Identification of 1,2,3,4,5,6-hexabromocyclohexane as a small molecule inhibitor of jak2 tyrosine kinase autophosphorylation [correction of autophosphorylation]. *J Med Chem* 48(7): 2526-33.
- Schwartz, Y., Yamaguchi, H. and Goodman, H. M. (1992). Growth hormone increases intracellular free calcium in rat adipocytes: correlation with actions on carbohydrate metabolism. *Endocrinology* 131(2): 772-8.
- Sekine, N., Ullrich, S., Regazzi, R., Pralong, W. F. and Wollheim, C. B. (1996). Postreceptor signalling of growth hormone and prolactin and their effects in the differentiated insulin-secreting cell line, INS-1. *Endocrinology* 137(5): 1841-50.
- Sencer, S., Papineni, R. V., Halling, D. B., Pate, P., Krol, J., Zhang, J. Z. and Hamilton, S. L. (2001). Coupling of RYR1 and L-type calcium channels via calmodulin binding domains. *J Biol Chem* 276(41): 38237-41.
- Sesti, G. (2002). Apoptosis in the beta cells: cause or consequence of insulin secretion defect in diabetes? *Ann Med* 34(6): 444-50.
- Silva, C. M., Day, R. N., Weber, M. J. and Thorner, M. O. (1993). Human growth hormone (GH) receptor is characterized as the 134-kilodalton tyrosine-phosphorylated protein activated by GH treatment in IM-9 cells. *Endocrinology* 133(5): 2307-12.
- Sirotkin, A. V. (2005). Control of reproductive processes by growth hormone: extra- and intracellular mechanisms. *Vet J* 170(3): 307-17.
- Sirotkin, A. V. and Makarevich, A. V. (1999). GH regulates secretory activity and apoptosis in cultured bovine granulosa cells through the activation of the cAMP/protein kinase A system. *J Endocrinol* 163(2): 317-27.
- Sirotkin, A. V. and Makarevich, A. V. (2002). Growth hormone can regulate functions of porcine ovarian granulosa cells through the cAMP/protein kinase A system. *Anim Reprod Sci* 70(1-2): 111-26.

- Sjoholm, A. (1996). Diabetes mellitus and impaired pancreatic beta-cell proliferation. *J Intern Med* 239(3): 211-20.
- Sjoholm, A., Zhang, Q., Welsh, N., Hansson, A., Larsson, O., Tally, M. and Berggren, P. O. (2000). Rapid Ca^{2+} influx and diacylglycerol synthesis in growth hormone-mediated islet beta -cell mitogenesis. *J Biol Chem* 275(28): 21033-40.
- Smal, J. and De Meyts, P. (1989). Sphingosine, an inhibitor of protein kinase C, suppresses the insulin-like effects of growth hormone in rat adipocytes. *Proc Natl Acad Sci U S A* 86(12): 4705-9.
- Smit, L. S., Meyer, D. J., Billestrup, N., Norstedt, G., Schwartz, J. and Carter-Su, C. (1996). The role of the growth hormone (GH) receptor and JAK1 and JAK2 kinases in the activation of Stats 1, 3, and 5 by GH. *Mol Endocrinol* 10(5): 519-33.
- Sneyd, J. and Falcke, M. (2005). Models of the inositol trisphosphate receptor. *Prog Biophys Mol Biol* 89(3): 207-45.
- Sorin, B., Vacher, A. M., Djiane, J. and Vacher, P. (2000). Role of protein kinases in the prolactin-induced intracellular calcium rise in Chinese hamster ovary cells expressing the prolactin receptor. *J Neuroendocrinol* 12(9): 910-8.
- Stofega, M. R., Herrington, J., Billestrup, N. and Carter-Su, C. (2000). Mutation of the SHP-2 binding site in growth hormone (GH) receptor prolongs GH-promoted tyrosyl phosphorylation of GH receptor, JAK2, and STAT5B. *Mol Endocrinol* 14(9): 1338-50.
- Stofega, M. R., Wang, H., Ullrich, A. and Carter-Su, C. (1998). Growth hormone regulation of SIRP and SHP-2 tyrosyl phosphorylation and association. *J Biol Chem* 273(12): 7112-7.
- Sudhof, T. C., Newton, C. L., Archer, B. T., 3rd, Ushkaryov, Y. A. and Mignery, G. A. (1991). Structure of a novel InsP_3 receptor. *Embo J* 10(11): 3199-206.
- Suko, J., Maurer-Fogy, I., Plank, B., Bertel, O., Wyskovsky, W., Hohenegger, M. and Hellmann, G. (1993). Phosphorylation of serine 2843 in ryanodine receptor-calcium release channel of skeletal muscle by cAMP-, cGMP- and CaM-dependent protein kinase. *Biochim Biophys Acta* 1175(2): 193-206.
- Sutko, J. L. and Airey, J. A. (1996). Ryanodine receptor Ca^{2+} release channels: does diversity in form equal diversity in function? *Physiol Rev* 76(4): 1027-71.
- Tam, S. P., Lau, P., Djiane, J., Hilton, D. J. and Waters, M. J. (2001). Tissue-specific induction of SOCS gene expression by PRL. *Endocrinology* 142(11): 5015-26.
- Wang, G. L., Shi, X., Salisbury, E., Sun, Y., Albrecht, J. H., Smith, R. G. and Timchenko, N. A. (2007). Growth hormone corrects proliferation and transcription of phosphoenolpyruvate carboxykinase in livers of old mice via elimination of CCAAT/enhancer-binding protein alpha-Brm complex. *J Biol Chem* 282(2): 1468-78.
- Waters, M. J., Hoang, H. N., Fairlie, D. P., Pelekanos, R. A. and Brown, R. J. (2006). New insights into growth hormone action. *J Mol Endocrinol* 36(1): 1-7.
- Weinhaus, A. J., Stout, L. E. and Sorenson, R. L. (1996). Glucokinase, hexokinase, glucose transporter 2, and glucose metabolism in islets during pregnancy and prolactin-treated islets in vitro: mechanisms for long term up-regulation of islets. *Endocrinology* 137(5): 1640-9.
- Weir, G. C., Laybutt, D. R., Kaneto, H., Bonner-Weir, S. and Sharma, A. (2001). Beta-cell adaptation and decompensation during the progression of diabetes. *Diabetes* 50 Suppl 1: S154-9.

- Yakar, S., Liu, J. L., Fernandez, A. M., Wu, Y., Schally, A. V., Frystyk, J., Chernausk, S. D., Mejia, W. and Le Roith, D. (2001). Liver-specific igf-1 gene deletion leads to muscle insulin insensitivity. *Diabetes* 50(5): 1110-8.
- Yip, R. G. and Goodman, H. M. (1999). Growth hormone and dexamethasone stimulate lipolysis and activate adenylyl cyclase in rat adipocytes by selectively shifting Gi alpha2 to lower density membrane fractions. *Endocrinology* 140(3): 1219-27.
- Yoshida, A., Ogura, A., Imagawa, T., Shigekawa, M. and Takahashi, M. (1992). Cyclic AMP-dependent phosphorylation of the rat brain ryanodine receptor. *J Neurosci* 12(3): 1094-100.
- Zhang, F., Zhang, Q., Tengholm, A. and Sjöholm, A. (2006). Involvement of JAK2 and Src kinase tyrosine phosphorylation in human growth hormone-stimulated increases in cytosolic free Ca²⁺ and insulin secretion. *Am J Physiol Cell Physiol* 291(3): C466-75.
- Zhang, Q., Kohler, M., Yang, S. N., Zhang, F., Larsson, O. and Berggren, P. O. (2004). Growth hormone promotes Ca²⁺-induced Ca²⁺ release in insulin-secreting cells by ryanodine receptor tyrosine phosphorylation. *Mol Endocrinol* 18(7): 1658-69.
- Zhu, T., Goh, E. L., LeRoith, D. and Lobie, P. E. (1998). Growth hormone stimulates the formation of a multiprotein signaling complex involving p130(Cas) and CrkII. Resultant activation of c-Jun N-terminal kinase/stress-activated protein kinase (JNK/SAPK). *J Biol Chem* 273(50): 33864-75.