

GH Signalling in Pancreatic β -Cells

NOBUO SEKINE, CLAES B. WOLLHEIM*, AND TOSHIRO FUJITA

Fourth Department of Internal Medicine, University of Tokyo School of Medicine, Tokyo 112, Japan, and

*Division de Biochimie Clinique, Centre Médical Universitaire, Département de Médecine, University of Geneva, CH-1211 Geneva 4, Switzerland

Abstract. GH and its related peptide PRL are known to stimulate proliferation and insulin biosynthesis in pancreatic β -cells, and assumed to be involved in their functional maturation. We investigated signal transduction of GH and PRL in insulin-secreting cells using the differentiated rat insulinoma cell line, INS-1. In these cells, both hormones stimulated proliferation and DNA synthesis, increased viability, cellular metabolism and insulin content. GH induced cytosolic Ca^{2+} ($[\text{Ca}^{2+}]_i$) rises, which appear to be due to Ca^{2+} -influx through voltage-gated Ca^{2+} -channels. GH also promoted tyrosine phosphorylation of several proteins in INS-1 cells, one of which was identified as JAK2 tyrosine kinase. Moreover, GH caused changes in DNA binding of nuclear proteins to some interferon- γ -activated sites. Verapamil inhibited neither DNA synthesis nor JAK2 phosphorylation stimulated by GH, whereas a tyrosine kinase inhibitor, lavendustin A, blocked the mitogenic effect. Involvement of cAMP is also suggested because Rp-cAMPS, a competitive inhibitor of protein kinase A, abolished both $[\text{Ca}^{2+}]_i$ rises and DNA synthesis stimulated by GH. The effects of GH and PRL on $[\text{Ca}^{2+}]_i$, JAK2 phosphorylation and DNA binding of the STATs were virtually identical in INS-1 cells. Since both hormones failed to activate MAP kinase in these cells, it is strongly suggested that activation of the JAK-STAT pathway is the major signalling event for the mitogenic effects of GH and PRL in β -cells. It remains to be clarified whether the $[\text{Ca}^{2+}]_i$ rise mediates other effects of these hormones.

Key words: GH, Pancreatic β -cells, Cytosolic Ca^{2+} , Tyrosine phosphorylation, The JAK-STAT pathway
(Endocrine Journal 45: S33–S40, 1998)

GH and PRL as Growth/Differentiation Factor for Pancreatic β -Cells

Patients with non-insulin-dependent diabetes mellitus (NIDDM) show impairment both in insulin secretion (insulin deficiency) and in insulin action (insulin resistance) [1]. Although the primary cause of NIDDM remains to be a controversial issue, it can be said that hyperglycemia develops when insulin secretory capacity fails to compensate for insulin resistance, either because of inadequate β -cell mass or because of decreased responsiveness of the β -cell to glucose stimulation. It is therefore

important to study the mechanisms regulating growth and the differentiated function of β -cells.

A variety of factors have been reported to stimulate replication of β -cells (Table 1) [2]. Among them, GH and its related peptides, PRL and placental lactogen [3], are especially of interest and their effects in insulin-secreting cells have been extensively studied. Table 2 summarizes the reported actions of GH-related hormones in various types of insulin-secreting cells. In addition to their mitogenic effect [4–11], these hormones are known to stimulate insulin biosynthesis [6, 9, 12–14], to promote oxidative metabolism of glucose [7, 9], and to increase GLUT2 expression [15], thereby contributing to the functional maturation (increase in sensitivity to glucose for the stimulation of insulin secretion) of β -cells [14–16]. In view of these various effects of GH-related peptides in β -

Correspondence to: Dr. Nobuo SEKINE, Fourth Department of Internal Medicine, University of Tokyo School of Medicine, 3–28–6 Mejirodai, Bunkyo-ku, Tokyo 112, Japan

Table 1. Reported mitogenic factors for insulin-secreting cells

Nutrients	Glucose, Amino acids, Fatty acids
Insulin-related factors	Insulin, IGF-I, IGF-II
The GH family	GH, PRL, placental lactogen
Growth factors	PDGF, EGF, bFGF, HGF
Gastrointestinal peptides	Gastrin, Cholecystokinin
Pharmacological agents	cAMP analogus*, Forskolin, Phorbol ester, Sulfonylureas*, Lithium, Nicotinamide

*contradictory.

Table 2. Actions of GH and PRL reported in various types of insulin-secreting cells

	GH	PRL
Proliferation/DNA synthesis ↑	islets (adult [4, 9], neonatal [7]) neonatal β -cells [5, 8] RIN-5AH cells [6]	islets (adult [4, 10, 11], neonatal [7, 10])
Insulin biosynthesis ↑	islets (adult [9], fetal [12]) RIN-5AH cells [6, 13]	islets (neonatal [14])
IGF-I production ↑	islets (adult [9], fetal [12]) human fetal pancreas [36]	(–)
Glucose oxidation ↑	islets (adult [9])	islets (neonatal [7])
Functional maturation	human fetal islet-like clusters [16]	islets (neonatal [14, 15])

References are indicated in parentheses.

cells, to study their mode of action might reveal crucial mechanisms for the regulation of growth and the function of these cells, which are only partially understood [2, 17].

The great advances in recent years in the study of growth factor/cytokine signalling have identified a variety of signalling events to be involved in the effects of GH, including the JAK-STAT pathway (see below), the mitogen-activated protein (MAP) kinase cascade, insulin receptor substrate (IRS)-1, IRS-2, phosphatidylinositol 3'-kinase, the generation of diacylglycerol followed by the activation of protein kinase C, and rises in cytosolic Ca^{2+} ($[\text{Ca}^{2+}]_i$) [Reviewed in 18]. However, little is known as to whether any of these mechanisms is involved in the actions of GH on β -cells. We therefore investigated effects and postreceptor signalling of GH and PRL using the differentiated insulin-secreting cell line INS-1 [19]. These cells retain a number of differentiated features of the native β -cell [19, 20] and express functional receptors for both GH and PRL [21].

The biological actions of GH and PRL were studied in INS-1 cells cultured in a defined serum-

free medium [22]. After 3 days or 7 days of culture, both 5 nM human GH (hGH) and 5 nM ovine PRL significantly stimulated proliferation and increased viability assessed by the MTT colorimetric assay (Fig. 1A). It should be noted that MTT reduction expressed per DNA reflects cellular oxidative metabolism in INS-1 cells [20] and that this value was indeed higher after 3 or 7 days culture with either hormone. These results suggest that both GH and PRL stimulate glucose metabolism in these cells. Cellular insulin content showed no difference among the three culture conditions after 3 days, whereas after 7 days, there was a marked decrease in the control (Fig. 1A). Fig. 1B shows the dose-response characteristics for the effect of GH and PRL on DNA synthesis assessed by [^3H]thymidine incorporation. hGH and PRL stimulated DNA synthesis in a dose-dependent manner in the physiological range (1 pM–1 nM). Bovine GH (bGH) was also tested because it binds specifically to GH receptors, whereas hGH could bind to PRL receptors as well as GH receptors [23]. The potency of these three hormones in the stimulation of DNA synthesis was virtually identical (Fig. 1B).

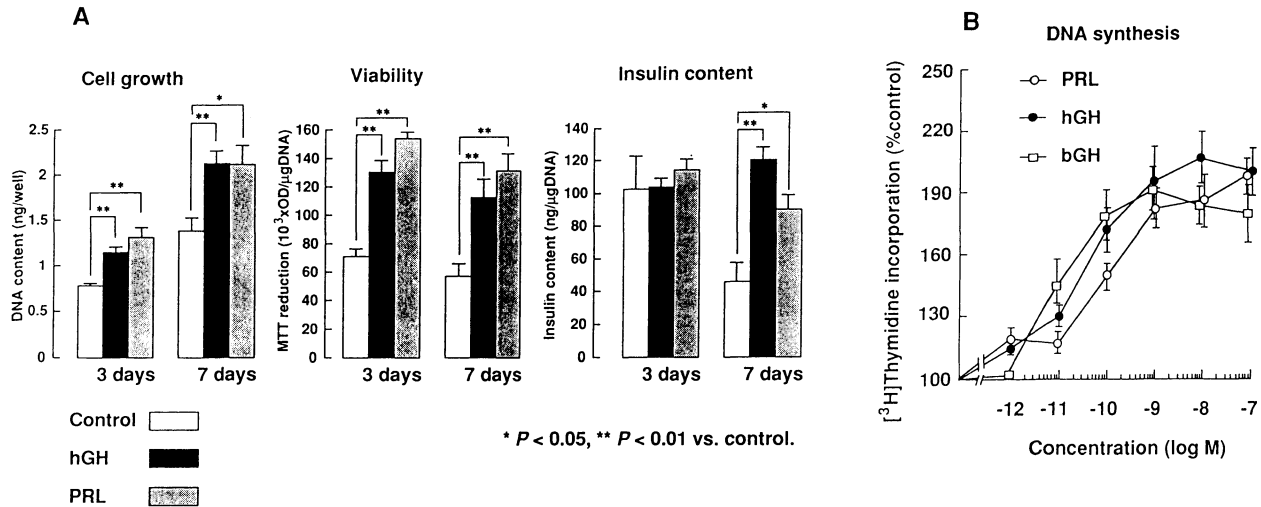


Fig. 1. Effects of GH and PRL on cell growth, viability, cellular insulin content (A), and DNA synthesis (B) of INS-1 cells. (A) Cells (2×10^4 cells/well in 96-well microtiter plates) were cultured in a defined serum-free medium in the absence or presence of 5 nM hGH or 5 nM PRL for 3 days and 7 days. Cell growth was evaluated by fluorimetric measurement of DNA content per well. The MTT colorimetric assay was performed as previously described [22]. (B) Cells (2×10^4 cells/well in 96-well microtiter plates) were incubated for 48 h in the serum-free medium containing various concentrations of hGH, bGH and PRL, followed by further 24-h incubation with the hormones in the presence of 0.5 μCi /well of $[\text{H}]$ thymidine, of which incorporation into the cells was measured with a liquid scintillation counter. Values are mean $\pm$ SEM from three independent experiments. *, $P < 0.05$, **, $P < 0.01$ vs. control, analyzed by one-way ANOVA. Modified from Sekine *et al.* [22]

GH is therefore a potent mitogen for INS-1 cells and is capable of maintaining their viability, oxidative metabolism and insulin biosynthesis. All of these effects observed in INS-1 cells are consistent with the findings reported in native β -cells (Table 2), indicating that this cell line provides a useful model to investigate GH signalling in β -cells.

The Ca^{2+} -Raising Effects of GH in Insulin-Secreting Cells

The change in cytosolic Ca^{2+} ($[\text{Ca}^{2+}]_i$), one of the major intracellular signalling events, is often associated with other mechanisms, such as phosphoinositide turnover, protein kinase C, adenylyl cyclase and G-proteins [24]. GH has also been reported to cause an increase in $[\text{Ca}^{2+}]_i$ in some, though not all, systems [18] including insulin-secreting cells [25, 26]. We therefore attempted to further characterize the $[\text{Ca}^{2+}]_i$ -raising effect of GH in INS-1 cells [22]. Changes in $[\text{Ca}^{2+}]_i$ were monitored by means of a microfluorimeter in single cells loaded with fura-2.

Representative traces obtained by this method are shown in Fig. 2. The addition of 5 nM GH

caused transient $[\text{Ca}^{2+}]_i$ oscillations both at 3 mM (Fig. 2A) and at 15 mM glucose (Fig. 2B). This effect was abolished by the Ca^{2+} -channel blockers, verapamil (Fig. 2C), and calciseptine (not shown) that blocks more specifically the L-type voltage-dependent Ca^{2+} -channels than verapamil. In fact, when diazoxide was added to hyperpolarize the membrane, the GH-induced $[\text{Ca}^{2+}]_i$ increase was no longer observed. Interestingly, Rp-cAMPS, a competitive inhibitor of protein kinase A, also abolished the effect of GH, without affecting glucose-induced $[\text{Ca}^{2+}]_i$ rises (Fig. 2D). The inhibitory effect of Rp-cAMPS on cAMP generation was confirmed, since the rise in $[\text{Ca}^{2+}]_i$ induced by glucagon-like peptide-1, which increases cAMP levels in INS-1 cells, was also blocked. Identical results were obtained with PRL in these cells.

The effects of GH on $[\text{Ca}^{2+}]_i$ in INS-1 cells are similar to those demonstrated in other types of insulin-secreting cells, fetal rat islet cells [25] and RIN-5AH cells expressing exogenous GH receptors [26], especially in that they are dependent on the presence of extracellular Ca^{2+} , and inhibited by the L-type Ca^{2+} -channel blockers. These findings indicated that the rise in $[\text{Ca}^{2+}]_i$ induced by GH in pancreatic β -cells are due to Ca^{2+} -influx through

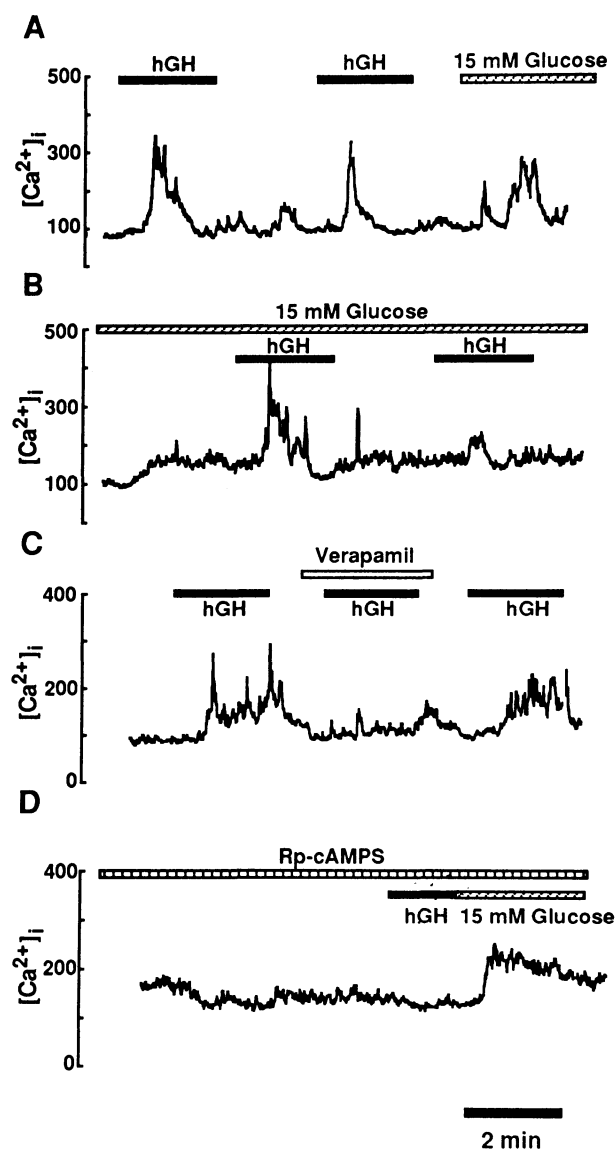


Fig. 2. Changes in cytosolic Ca^{2+} ($[Ca^{2+}]_i$) by GH in INS-1 cells. The effects of 5 nM hGH at 3 mM glucose in the absence (A) or presence of 20 μ M verapamil (C) or 1 mM Rp-cAMPS (D), and at 15 mM glucose (B) are shown. Single cells on glass coverslips were loaded with 1 μ M fura-2/AM for 30 min. The change in $[Ca^{2+}]_i$ was monitored by the measurement of the 340/380 ratio in a microfluorimeter system [22].

voltage-dependent Ca^{2+} -channels, and prompted us to perform electrophysiological studies to examine whether GH in fact causes membrane depolarization. GH caused membrane depolarization in INS-1 cells, although this effect was observed only in a small proportion (<25%) of

the cells, and not detected in a cell population by the fluorescence method with bisoxonol as probe [22]. The reason that only a minor proportion of the cells responded with membrane depolarization is unclear. Nevertheless, it could be said at least that a certain level of membrane potential is crucial for GH to elicit its $[Ca^{2+}]_i$ -raising effect. It remains to be clarified whether GH-induced Ca^{2+} -influx is secondary to other signalling events, and whether this event is associated with [27], or independent of [26] JAK2 activation (see below).

Activation of the JAK-STAT Pathway by GH and PRL in INS-1 Cells

Both GH and PRL belong to the cytokine/hematopoietic growth factor receptor superfamily [3, 28]. Recently much attention has been directed to the JAK-STAT pathway [29], which is now established as the major event for cytokine signalling. As is the case with a number of cytokines, both GH and PRL phosphorylate and activate JAK2 tyrosine kinase associated with their receptors, leading to activation of a family of transcription factors STAT (Signal Transducers and Activators of Transcription), which bind to the DNA to promote transcription [18, 30 and references therein].

Little information is available, however, on the JAK-STAT pathway in insulin-secreting cells. We therefore investigated activation of this pathway by GH and PRL in INS-1 cells. We first examined tyrosine phosphorylation of cellular proteins by Western blotting with the antibody against phosphotyrosine. After a 5-min incubation in the presence of 5 nM GH, several proteins (e.g. of about 180, 130, and 90 kDs) were found to be tyrosine phosphorylated (Fig. 3A). The phosphorylated protein of about 130 kD was identified as JAK2 by immunoprecipitation with anti-JAK2 serum (Fig. 3B). When verapamil was added during the incubation, phosphorylation of the proteins by GH (Fig. 3A), including that of JAK2 (Fig. 3B), was not affected. Glucose and fetal calf serum, both of which stimulate proliferation of INS-1 cells [31], failed to stimulate JAK2 phosphorylation (Fig. 3B). It is possible that their effects are mediated by the MAP kinase pathway, that is indeed activated by these factors in INS-1 cells [31].

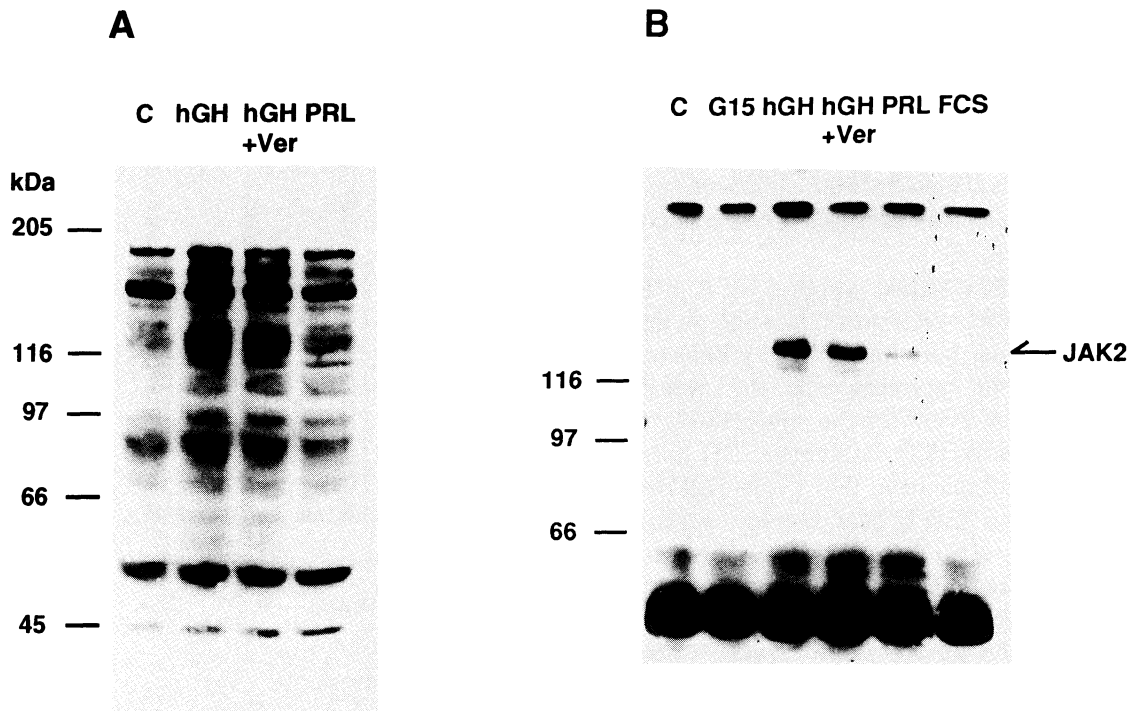


Fig. 3. Tyrosine phosphorylation of cellular proteins (A) and JAK2 (B) promoted by GH in INS-1 cells. After overnight incubation in a serum-free medium, cells were preincubated for 30 min without stimuli, and stimulated for 5 min with factors as indicated. Total cell lysates (A) or immunoprecipitates after incubation with anti-JAK2 serum (B, lower panel) were immunoblotted with antibodies against phosphotyrosine. In (B, upper panel), the same nitrocellulose membrane as used in B, lower panel was immunoblotted with anti-JAK2 serum to show the presence of JAK2 protein in every lane. Ver, verapamil (20 μ M); G15, 15 mM glucose; FCS, fetal calf serum. Modified from Sekine *et al.* [22].

It has already been reported in other systems that GH activates three STATs, namely STAT1, STAT3 and STAT5 [18]. It is, however, not known which STAT is virtually activated in insulin-secreting cells to mediate the effects of GH or PRL. This question seems to be crucial, since activation of STATs might determine the tissue-specific mode of action of GH.

Electrophoretic mobility shift assay was performed to investigate DNA binding of the STATs. Three oligonucleotides representing distinct interferon (IFN)- γ -activated sites (GAS) were synthesized for IFN-regulatory factor 1 (IRF-1) promotor (IRF-1 GAS) [32], *sis*-inducible element in the *c-fos* promotor gene (SIE) [33], and β -casein gene promotor (β -CAS GAS) [34]. The IRF-1 GAS was first identified as the DNA binding site for STAT1 activated by IFN- γ and was later shown to be that of STAT5 activated by PRL [32]. Both STAT1 and STAT3 have been shown to bind to SIE, whereas STAT5 preferentially binds to β -CAS GAS. After 15-min stimulation with GH or PRL,

DNA binding of nuclear proteins to β -CAS GAS was significantly increased, but that to SIE showed no significant change. Interestingly, DNA-binding to IRF-1 GAS was rather decreased by both hormones (Sekine N, Ishikawa T, Okazaki T, Fujita T, unpublished observations). Implications of these preliminary findings are under investigation in our laboratory.

Signalling Mechanisms Mediating the Mitogenic Effect of GH and PRL in INS-1 Cells

Finally, in order to know how the signalling events described above are implicated in the mitogenic effect of GH, we performed studies with [^{3} H]thymidine in the presence of various inhibitors. We used verapamil to block Ca^{2+} -influx, diazoxide to prevent membrane depolarization, Rp-cAMPS to inhibit protein kinase A, and lavendustin A to inhibit tyrosine kinases. Neither verapamil nor

diazoxide affected stimulation of DNA synthesis by GH (Fig. 4). Therefore, at least for the mitogenic effect, the rise in $[Ca^{2+}]_i$ is not required, whereas involvement of cAMP is suggested because Rp-cAMPS clearly inhibited the effect of GH. Finally, lavendustin A abolished the mitogenic effect of GH, indicating that tyrosine phosphorylation is crucial for this effect (Fig. 4).

It remains to be clarified whether the $[Ca^{2+}]_i$ rise is involved in other effects of GH, such as insulin biosynthesis as has been suggested by Billestrup *et al.* [26]. In insulin-secreting cells, the increase in $[Ca^{2+}]_i$ is a crucial event for the stimulus-secretion coupling of insulin [35]. However, GH failed to induce insulin release from INS-1 cells [22] despite the clear effect on $[Ca^{2+}]_i$. It is therefore speculated that some inhibitory mechanisms on insulin secretion are concomitantly activated by GH.

Recent investigations revealed that GH also activates the MAP kinase pathway, which has also been implicated in GH signalling in several other systems [18]. In INS-1 cells, however, it has already been shown that neither GH nor PRL stimulates the MAP kinase pathway [31]. Moreover, some factors that are capable of stimulating this pathway, such as nerve growth factor or cAMP agonists, do not elicit mitogenic effect in these cells. Taken together, these findings strongly suggest that the JAK-STAT pathway is the major signalling event mediating the mitogenic effect of GH.

Acknowledgements

The authors thank Drs Isabelle Borghini, Romano Regazzi, and William-F. Pralong (Division de Biochimie Clinique, Centre Médical Universitaire, University of Geneva) for valuable support and useful discussion, Susanne Ullrich (Albert-Ludwigs-Universität, Freiburg/Br, Germany) for performing electrophysiological studies, and Drs Toshio Ishikawa and Tomoki Okazaki (Fourth Department

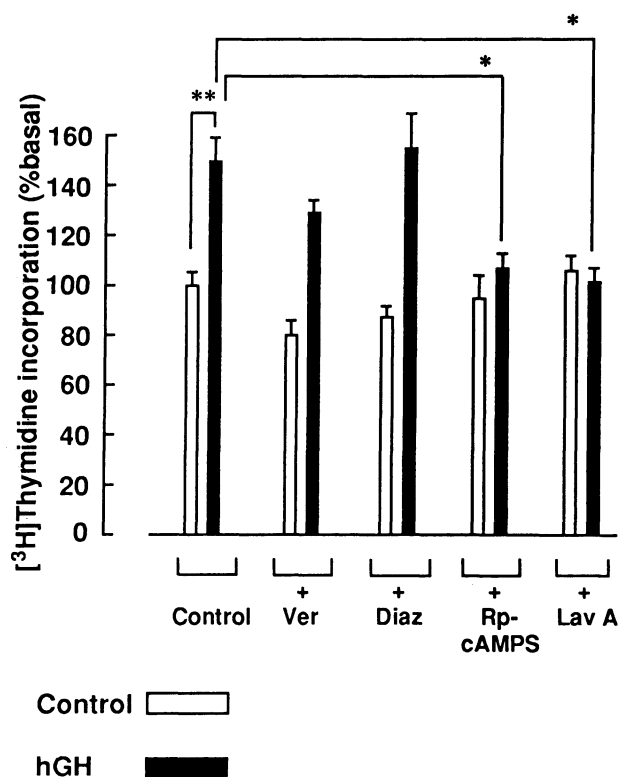


Fig. 4. Effects of various inhibitors on GH-stimulated DNA synthesis in INS-1 cells. Cells were stimulated with 5 nM hGH in the absence or presence of inhibitors for 6 h, and then washed and incubated without stimuli or inhibitors for 18 h. $[^3H]$ Thymidine was present during the final 4-h period of incubation. Ver, verapamil (20 μ M); Diaz, diazoxide (200 μ M); Rp-cAMPS (1 mM); Lav A, lavendustin A (1 μ M). Values are mean $\pm$ SEM from three independent experiments. *, $P < 0.05$, **, $P < 0.01$ vs. control, analyzed by Mann-Whitney *U* test. Modified from Sekine *et al.* [22].

of Internal Medicine, University of Tokyo) for indispensable help. This work was supported by grants from the Swiss National Science Foundation 32-32376.91 (to C.B.W.), by Ares Serono (Geneva, Switzerland) and by the Helmut-Horten Foundation (Madonna del Piano, Switzerland).

References

1. Reaven GM (1988) Banting Lecture 1988: Role of insulin resistance in human disease. *Diabetes* 37: 1595-1607.
2. Swenne I (1992) Pancreatic beta-cell growth and diabetes mellitus. *Diabetologia* 35: 193-201.
3. Kelly PA, Ali S, Rozakis M, Goujon L, Nagano M, Pellegrini I, Gould D, Djiane J, Edery M, Finidori J, Postel-Vinay MC (1993) The growth hormone/prolactin receptor family. *Rec Prog Horm Res* 48: 123-164.

4. Nielsen JH (1982) Effects of growth hormone, prolactin, and placental lactogen on insulin content and release, and deoxyribonucleic acid synthesis in cultured pancreatic islets. *Endocrinology* 110: 600–606.
5. Rabinovitch A, Quigley C, Rechler MM (1983) Growth hormone stimulates islet β -cell replication in neonatal rat pancreatic monolayer cultures. *Diabetes* 32: 307–312.
6. Billestrup N, Martin JM (1985) Growth hormone binding to specific receptors stimulates growth and function of cloned insulin-producing rat insulinoma RIN-5AH cells. *Endocrinology* 116: 1175–1181.
7. Brelje TC, Allaire P, Hegre O, Sorenson RL (1989) Effect of prolactin *versus* growth hormone on islet function and the importance of using homologous mammosomatotropic hormones. *Endocrinology* 125: 2392–2399.
8. Nielsen JH, Linde S, Welinder BS, Billestrup N, Madsen OD (1989) Growth hormone is a growth factor for the differentiated pancreatic β -cell. *Mol Endocrinol* 13: 165–173.
9. Swenne I, Hill DJ (1989) Growth hormone regulation of DNA replication, but not insulin production, is partly mediated by somatomedin-C/insulin-like growth factor I in isolated pancreatic islets from adult rats. *Diabetologia* 32: 191–197.
10. Brelje TC, Sorenson RL (1991) Role of prolactin *versus* growth hormone on islet B-cell proliferation *in vitro*: Implications for pregnancy. *Endocrinology* 128: 45–57.
11. Sorenson RL, Brelje TC, Roth C (1993) Effects of steroid and lactogenic hormones on islets of Langerhans: A new hypothesis for the role of pregnancy steroids in the adaptation of islets to pregnancy. *Endocrinology* 133: 2227–2234.
12. Swenne I, Hill DJ, Strain AJ, Milner RDG (1987) Growth hormone regulation of somatomedin C/insulin-like growth factor I production and DNA replication in fetal rat islets in tissue culture. *Diabetes* 36: 288–294.
13. Billestrup N, Møldrup A, Serup P, Mathews LS, Norstedt G, Nielsen JH (1990) Introduction of exogenous growth hormone receptors augments growth hormone-responsive insulin biosynthesis in rat insulinoma cells. *Proc Natl Acad Sci USA* 87: 7210–7214.
14. Boschero AC, Crepaldi SC, Carneiro EM, Delattre E, Atwater I (1993) Prolactin induces maturation of glucose sensing mechanism in cultured neonatal rat islets. *Endocrinology* 133: 515–520.
15. de Mazancourt P, Carneiro EM, Atwater I, Boschero AC (1994) Prolactin treatment increases GLUT2 but not the G protein subunit content in cell membranes from cultured neonatal rat islets. *FEBS Lett* 343: 137–140.
16. Otonkoski T, Knip M, Wong I, Simell O (1988) Effects of growth hormone and insulin-like growth factor I on endocrine function of human fetal islet-like cell clusters during long-term tissue culture. *Diabetes* 37: 1678–1683.
17. Welsh M, Mares J, Öberg C, Karlsson T (1993) Genetic factors of importance for β -cell proliferation. *Diabetes Metab Rev* 9: 25–36.
18. Argetsinger LS, Carter-Su C (1996) Mechanisms of signaling by growth hormone receptor. *Physiol Rev* 76: 1089–1107.
19. Asfari M, Janjic D, Meda P, Li G, Halban PA, Wollheim CB (1992) Establishment of 2-mercaptoethanol-dependent differentiated insulin-secreting cell lines. *Endocrinology* 130: 167–178.
20. Sekine N, Cirulli V, Regazzi R, Brown LJ, Gine E, Tamarit-Rodriguez J, Girotti M, Marie S, MacDonald MJ, Wollheim CB, Rutter GA (1994) Low lactate dehydrogenase and high mitochondrial glycerol phosphate dehydrogenase in pancreatic β -cells: Potential role in nutrient sensing. *J Biol Chem* 269: 4895–4902.
21. Asfari M, De W, Postel-Vinay M-C, Czernichow P (1995) Expression and regulation of growth hormone (GH) and prolactin (PRL) receptors in a rat insulin producing cell line (INS-1). *Mol Cell Endocrinol* 107: 209–214.
22. Sekine N, Ullrich S, Regazzi R, Pralong W-F, Wollheim CB (1996) Postreceptor signalling of growth hormone and prolactin and their effects in the differentiated insulin-secreting cell line, INS-1. *Endocrinology* 137: 1841–1850.
23. Ranke MB, Stanley CA, Tenore A, Rodbard D, Bongiovanni AM, Parks JS (1976) Characterization of somatogenic and lactogenic binding sites in isolated rat hepatocytes. *Endocrinology* 99: 1033–1045.
24. Clapham DE (1995) Calcium signaling. *Cell* 80: 259–268.
25. Sjöholm Å, Zhang Q, Welsh N, Öberg C, Tally M, Berggren P-O (1994) Growth hormone causes a rapid, sustained increase in Ca^{2+} influx, diacylglycerol formation in pancreatic islet β -cells. *Diabetologia* 37 (Suppl 1): A32 (Abstract).
26. Billestrup N, Bouchelouche P, Allevato G, Ilondo M, Nielsen JH (1995) Growth hormone receptor C-terminal domains required for growth hormone-induced intracellular free Ca^{2+} oscillations and gene transcription. *Proc Natl Acad Sci USA* 92: 2725–2729.
27. Prevarskaya NB, Skryma RN, Vacher P, Daniel N, Djiane J, Dufy B (1995) Role of tyrosine phosphorylation in potassium channel activation: Functional association with prolactin receptor and JAK2 tyrosine kinase. *J Biol Chem* 270: 24292–24299.
28. Horseman ND, Yu-Lee L-Y (1994) Transcriptional regulation by the helix bundle peptide hormones: Growth hormone, prolactin, and hematopoietic cytokines. *Endocr Rev* 15: 627–649.

29. Schindler C, Darnell Jr JE (1995) Transcriptional responses to polypeptide ligands: The JAK-STAT pathway. *Annu Rev Biochem* 64: 621–651.
30. Leaman DW, Leung S, Li X, Stark GR (1996) Regulation of STAT-dependent pathway by growth factors and cytokines. *FASEB J* 10: 1578–1588.
31. Frödin M, Sekine N, Roche E, Filloux C, Prentki M, Wollheim CB, van Obberghen E (1995) Glucose, other secretagogues, and nerve growth factor stimulate mitogen-activated protein kinase in the insulin-secreting β -cell line, INS-1. *J Biol Chem* 270: 7882–7889.
32. Stevens AM, Wang Y-f, Sieger KA, Lu H-f, Yu-Lee L-y (1995) Biphasic transcriptional regulation of the interferon regulatory factor-1 gene by prolactin: Involvement of γ -interferon-activated sequence and Stat-related proteins. *Mol Endocrinol* 9: 513–525.
33. Gronowski AM, Zhong Z, Wen Z, Thomas MJ, Darnell Jr JE, Rotwein P (1995) *In vitro* growth hormone treatment rapidly stimulates the tyrosine phosphorylation and activation of Stat3. *Mol Endocrinol* 9: 171–177.
34. Tourkine N, Schindler C, Larose M, Houdebine L-M (1995) Activation of STAT factors by prolactin, interferon- γ , growth hormones, and a tyrosine phosphatase inhibitor in rabbit primary mammary epithelial cells. *J Biol Chem* 270: 20952–20961.
35. Wollheim CB, Sharp GWG (1981) Regulation of insulin release by calcium. *Physiol Rev* 61: 914–973.
36. Swenne I, Hill DJ, Strain AJ, Milner RDG (1987) Effects of human placental lactogen and growth hormone on the production of insulin and somatomedin C/insulin-like growth factor I by human fetal pancreas in tissue culture. *J Endocrinol* 113: 297–303.